ULTRASTRUCTURE OF SELECTED FLAGELLATED CHRYSOPHYTES

by 239E

Daniel E. Wujek
B.S., Central Michigan University, 1961
M.A., Central Michigan University, 1962

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Chairman

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INTRODUCTION

The Chrysophyceae is a class within the algal Division Chrysophyta. The Division also includes the classes Xanthophyceae, Bacillariophyceae and Haptophyceae. There is a serious lack of detailed knowledge about the structure and life history of the members of the Chrysophyceae, and surprisingly little is known about their occurrence and distribution in the United States. Even in the comparatively well explored lakes of the midwest there is a paucity of information about the class (Smith, 1920; Prescott, 1964). However, a rich chrysophycean flora occupying a broad range of habitats in Minnesota and eastern Kansas and the widespread, and at times abundant occurrence of these algae has provided the stimulus for the present investigation.

Previous work on the Chrysophyceae has dealt principally with the morphology of the vegetative cell, and it is these studies which have furnished basic taxonomic information and resulted in an extensive literature. Only recently have there been a few investigations into the fine structure of these organisms so our knowledge of their ultrastructure is still very meager.

This study represents a detailed examination of the fine structure of the families Mallomonadaceae and Ochromonadaceae (Smith, 1950). The species selected exemplify a diversity of types found within the two families.

Basic cell fine structure and the character of the cytoplasmic organelles are examined in this study. Knowledge of these now makes it possible to establish a more exact descriptive morphology. The study also provides comparative ultrastructural evidence bearing on the systematic treatments proposed by Fritsch (1935), Fott (1959), Smith (1950), and Bourrelly (1957).

MATERIALS AND METHODS

The species examined in this investigation were taken from natural populations and the writer's culture collection. Species examined from their natural environment were Chrysophaerella itascaei Thomp. and Wujek, Microglena punctifera (O.F. Mull.) Ehrenb., (Mallomonadaceae), Uroglenopsis sp., Uroglenopsis notabilis Mack, Uroglena sp., Uroglena collaris Thomp., Dinobryon sertularia Ehrenb., Dinobryon divergens Imhof., and Dinobryon bavaricum Lemm., (Ochromonadaceae). The species examined from cultures were Ochromonas thompsonii sp. nov., Epipyxis andrewsii sp. nov. and Epipyxis sp.

The cultures of Ochromonas and Epipyxis were maintained in soil-water medium in Petri dishes. The latter was prepared by steaming 500 grams of soil (collected from overlays of limestone and sandstone) in 1000 ml of water for one hour on two successive days. The supernatant was diluted with three or four parts of distilled water. Illumination was furnished by a north facing window.

Natural populations of the various species were collected in the Lake Itasca Park region, Minnesota, and in small ponds and pools in the vicinity of Lawrence, Kansas. Plankton tows (#25 mesh net) were used to concentrate the organisms from their natural environment. Fixation was started directly in the field at the same temperatures or the sample was transported to the laboratory in a water-jacketed container for fixation.

In the laboratory, the samples were further concentrated by gentle centrifugation. A variety of fixatives were used throughout this study: glutaraldehyde, osmium tetroxide, potassium permanganate, or a combination of these.

The permanganate fixative was a 2% concentration of potassium permangante in sodium veronal and sodium acetate, as recommended by Luft (1956), or in unbuffered deionized water. No differences in fixation could be observed between the buffered and unbuffered fixatives. Fixation time was 45 to 90 minutes, with the longer periods being preferred.

Osmium tetroxide fixation involved either a 2% unbuffered or 1% buffered solution (Palade, 1952). Fixation time was 30 to 90 minutes at room temperatures. The best morphological detail was obtained by fixing 60 to 70 minutes in 1% buffered osmium tetroxide in an ice water bath.

The glutaraldehyde was purified by mixing it with activated charcoal, 0.1 to 0.06% the volume of glutaraldehyde and then filtering. In fixation, either a 2 or 3% buffered 0.2M phosphate solution was used 30 to 90 minutes (Sabatini, Bensch and Barrnett, 1963). The tissue was then washed 3 times for 15 minutes each with a 0.2M phosphate solution buffered at the appropriate pH. PH was 6.8 to 7.4. Post-fixation was 1% buffered osmium tetroxide for 30 to 120 minutes.

Fixed material was rinsed in distilled water 1 or 2 minutes prior to dehydration. The tissue was dehydrated

through 25, 50, 75 and 95% ethyl alcohol or acetone for 10 to 15 minutes each. If alcohol was used, the 95% change was followed by 1 change of 100% ethyl alcohol and 2 changes in 100% propylene oxide for 10 to 15 minutes each. The acetone series ended with three 10 to 15 minute changes of 100% acetone.

Infiltration of Mollenhauer's (1964) epoxy resin #1 was initiated with a 1:2 mixture of resin and acetone respectively for at least 2 hours, ranging to 24 hours. If propylene oxide was used as the final change in dehydration, it was substituted for the acetone. At the end of this period the tissue was placed in a 2:1 mixture for another 2 hours, again ranging to 24 hours. At the end of this series, using DMP-30 as the catalyst, the tissue was placed in 100% epoxy resin for polymerization in aluminum foil boats for 24 fours at 60 to 75 C. Although some tissue was placed in a vacuum oven for polymerization, no differences could be observed in the vacuum oven compared with the non-vacuumed tissue.

After polymerization, a section of the embedded material was cut out and mounted on a 1.5 cm glass rod chuck with Eastman 910 Adhesive and trimmed (Pease, 1964).

All sections were cut with glass knives or a du Pont diamond knife mounted on a LKB Ultrotome or a Porter-Blum MT-1 microtome. Glass knives were made of 3/16 inch hard glass supplied through LKB Instruments. The method of

glass breaking was one using only free breaks along fracture lines of the glass. The knives were then fitted with a collection boat of black electrician's tape about one-quarter inch in width with a final seal of bees wax as described by Pease (Pease, 1964).

Sections were mounted directly on uncoated 200 or 400 mesh grids. The interference colors of the sections were either gold or silver when collected in 10% acetone or distilled water in the knife boat. For greater contrast, some sections were stained 10 to 20 minutes with Millonig's (1961) lead stain.

External morphology was studied in whole mounts of cells killed directly on prepared grids by a 30 second exposure to osmic vapors. Coated grids were prepared using Formvar or 2% parlodion; some were carbon stablized. Shadowcast whole mounts were made with platinium-palladium wire using a Kinney SC-3 STD evaporator. Direct prepreations of this kind were used to reveal the general form of the cell with its scales, spines and flagella.

Specimens were examined on an RCA EMU 3F2 electron microscope operated at 50kv with a 35-40μ objective aperture.

Micrographs were taken on 2 X 10 inch and 3¼ X 4 inch contrast grade Kodak lantern slides. These were developed in Kodak D-19 developer for 3 minutes, fixed in hypo for 5 to 10 minutes, and washed for 30 minutes. The washed plates were immersed in KOdak Photo-flo for 1 minute and air dried.

Prints from the micrographs were made on Agfa or Kodak enlarging papers of various grades.

Light micrographs were made on Kodak Pan-X film using an A. O. Spencer Microstar microscope fitted with a Kodak 35 mm back. Film was developed in Kodak Microdol for 7 to 9 minutes, fixed in hypo for 10 minutes, washed for 20 minutes and air dried.

Various cyto-chemical staining techniques were employed to test for the presence of pectin, cellulose, volutin, lignin, chrysolaminarin, etc. The presence of the pectin was tested with neutral violet and brilliant cresyl blue. The latter, along with methlene blue, was also used for chrysolaminarin and muciferous bodies. Lignin was tested for with methyl red.

DESCRIPTION OF THE LIGHT MICROSCOPY AND FINE STRUCTURE OF THE ORGANISMS STUDIED

Microglena Ehrenberg, 1838

The Chrysophyceaen alga, Microglena was first described by Muller (1786) under the name Enchelys punctifera. Ehrenberg (1838) described it under the genus Microglena. In 1927, Conrad monographed the genus which today includes seven species.

The genus Microglena is characterized by a firm envelope in which, according to Conrad (1927), one can distinguish a delicate inner, smooth layer composed of cellulose and an outer, thicker layer of pectic substances at the surface of which numerous lens-shaped masses of silica are embedded. Klebs (1892), Pascher (1913) and Deflandre (1932) also observed these silica particles.

Microglena punctifera was collected from a pond along Lakes Drive, one-quarter mile north of Deming Lake in Itasca State Park, Lake Itasca, Minnesota, in June and July of 1964. It occurred in a small pool in a marginal Sphagnum mat. Embedded silica granules were not evident on the periplast of this material (Fig. 1). The single anterior flagellum was about twice the body length. There were one or two chromatophores with a stigma at the anterior end of one, and the conspicuous nucleus was central in position. A large chrysolaminarin (leucosin) body was in the posterior

portion of the cell.

Electron microscopy:

A fixation routine that proved satisfactorly for other organisms in this study was inadequate for this species. Thus a detailed description of the ultrastructure of this organism was not made.

The only worthwhile observation made was that of the appearance of the embedded silica granules (Fig. 2). These were not observed under the light microscope. Lack of material also made it impossible to establish if these granules were silica as in the scales of Chrysophaerella.

Chrysophaerella Lauterborn, 1896

Colonies of Chrysophaerella are composed of a few to many (up to 100 and often more) radially arranged cells. Cells are pyriform, covered by an envelope bearing elliptic, smooth, silicified scales and up to six spines with furcate ends.

Originally, Chrysophaerella was described as possessing only a single flagellum. Korschokov (1941) noted the presence of a second, shorter flagellum. This observation has been overlooked by all recent systematic treatments of the genus aside from that of Skuja (1948). This will be considered in greater detail in a later section.

A large mass of chrysolaminarin is near the posterior end of each cell and below it are several contractile vacuoles.

Two biscuit-shaped chromatophores are in the anterior half of each cell. One of them bears a stigma and adjacent to this is the shorter flagellum. A large nucleus lies between the chromatophores. Cysts have been reported for one species.

Chrysophaerella itascaei (Fig. 3), in addition to the above characteristics possesses a variable number of spines. They are not limited to two, as is described in the diagnosis of the type species, Chrysophaerella longispina Lautb. Korschikov (1941) reported as many as five for C. longispina. According to my observations, there are more than two and may be as may as seven in C. itascaei.

Electron microscopy:

Scales and spines

The morphology and structure of the scales and spines have been described already (Thompson and Wujek, in preparation). To establish the siliceous nature of the scales and spines, an entire block was immersed in ten per cent hydrofluoric acid (HF) for 36 hours prior to sectioning (technique of Drum, 1963). Figure 4 shows that the normally opaque scales are dissolved with HF treatment. Other acids (HCl, H_2SO_4) had no observable effect on the scales or spines. This indicates that both scales and spines are made of silica.

Scale origin has been reported in other Chrysophytes, e.g.

Synura (Manton, 1955a), Paraphysomonas vestita (Manton and Leedale, 1961) and Chrysochromulina polylepis (Manton and Parke, 1962). All three investigations reported conflicting views as to scale origin. Manton and Leedale observed scale origin from vesicles within the cytoplasm, while Manton and Parke observed scale origin from the plasma membrane.

It has not been possible to follow all the developmental details in C. itascaei since scales and spines can scarcely be recognized as such in a very incomplete condition. The probable site of synthesis is however, in vesicles of the Golgi body as Manton et al. (1965) observed in Heteromastix. The vesicles in Fig. 5 are thought to be the ones that fuse and eventually form the scale. Proof that these vesicles contain silica can be seen in a HF treated section (Fig. 4). The membrane of the vesciles are still intact, but the particles are no longer evident. The vesicles then begin to fuse with one another and eventually a partial scale is formed (Fig. 6) as a result of a lemellation process. It therefore seems probable that the early stages are within these large vesicles and that the transfer of the scale to the surface is the final act (Figs. 7 and 8). Very few scales were cut in oblique transverse sections (Fig. 9).

Internal scales are by no means always encountered singly. Figures 10 and 11 show two sections with three apparent vesicles, each with a scale. Vesicles containing a

scale, such as those of Figs. 10 and 11, do not resemble food vacuoles (Figs. 12 and 13), although it should not be forgotten that this species is phagotrophic and some foreign scales could conceivabely be ingested during feeding.

Figure 14 shows a spine about to immerge from a cell. The residual vacuolar membrane surrounding the spine is still evident. The spine tip is still covered by the cell membrane which is still distorted though not yet punctured (Fig. 15).

It had been hoped that scale and spine development might have been traceable in living cells by means of phase-illumination, but the optical properties of the cytoplasm are such as to mask completely the presence of internal scales and spines.

Chromatophore

Observations of the ultrastructure reveals the presence of two plstids; such has been observed in the light microscope. The chromatophore is limited by a dense membrane. In favorable sections, this membrane is resovable into three membranes (Fig. 16). The outer membrane being a portion of the nuclear envelope.

It can be seen in Figs. 17 and 18 that the lamellae are arranged in groups of four, of which the inner two are thicker than the outer two. This has been reported by other Chrysophyte researchers (Drum, 1963, 1964; Gibbs, 1962a; Joyon, 1960, 1963; Stroemer and Pankratz, 1964). The fact

that the inner lamellae are approximately double the width of the thin outer lamellae suggests that the inner lamellae are two closely appressed or fused thin lamellae, and thus that a band of two thin lamellae and two thick lamellae is actually three discs. Proof of this comes only from careful tracing of the individual lamellae. Usually when a thick band splits, two thin lamellae are the result (Figs. 17 and 19).

The termination of the discs of the lamellae at the edge of the chromatophores are usually hard to see because they are closely appressed to the limiting membranes. However, in favorable sections, the discs of a band end before they reach the limiting membrane, and there the disc nature of the lamellae can be clearly determined. Figures 19 and 20 reveal how a band of four lamellae end as closed discs at the edge of the chromatophore.

The chromatophore matrix in which the bands of discs lie consists of an irregularly granular material. Scattered in this granular matrix are large lipid droplets. No pyrenoid could be observed.

Nucleus

The nucleus is the most important organelle to recognize at the outset, since until this is done in certain types of sections it could easily be mistaken for some cell component. In many sections it is the only major organ visible. It contains a single nucleolus, and the rest of the nuclear

volume is occupied by irregularly disposed light and dark masses. Differences in the overall shape and position undoubtedly change with cell metabolism and with fixation. Thus in Fig. 21, the nucleus is very asymmetrical both in shape and position while that of Fig. 18 is of a more normal appearance.

Consistently located between the plastid and the nucleus is a narrow space which contains a number of "circular profiles" (Figs. 22 and 23) as Gibbs (1962a, 1962b) has termed them.

Eyespot

The stigma of C. itascaei consists of a curved plate of many orange red granules (light microscopy) lying within the tip of one of the plastids (Fig. 24). This is in contrast to the eyespot of Euglena gracilis which lies in the cytoplasm close to the plastid, but not bounded by the chloroplast membrane (Gibbs, 1960).

Mitochondria

Numerous spherical and rod-shaped mitochondria are found throughout the cell limited by the characteristic double membrane. Of special interest is the mitochondrion which appears above the nucleus and between the two chromatophores (Fig. 25). It is present at the same position in all cells cut in a similar plane and is thought to have some relationship with the flagella as is observed in other

algae. As many as seven mitochondria have been observed in one section.

Fat bodies

Unsaturated fat bodies, easily identifiable as such by their opacity following fixation are of common occurrence. They are variable in size and not very numerous. Several are contained in Fig. 29.

Food vacuoles

Food vacuoles are distinguishable from other cytoplasmic vesicles by irregular contents which contain in most cases unrecognizable objects. Several food vacuoles with rather indefinite contents are present in Figs. 12 and 13. It has been suggested that scales may be ingested this way, but no food vacuoles containing recognizable objects such as newly ingested scales of other scaled Chrysophytes have been observed.

Golgi

Immediately below the attachment of the flagellar bases is an area occupied by the Golgi body. This Golgi area is commonly bounded by the nucleus, mitochondria and chromatophores (Fig. 18), but never separated from the flagellar bases (Fig. 26) by any of these organelles. Such a position has been termed perinuclear (Drum, 1964).

When cut tangentially or merely grazed, only the com-

pacted vesicles and traces of flattened paired membranes are visible (Fig. 26). When cut more nearly centrally, however, a surprising complexity of structure is revealed. Figures 6 and 27 are inserted to illustrate this. The Golgi consists of 4-15 tightly packed cisternae with slightly dialated rims.

Chrysolaminarin

Chrysolaminarin in osmium fixed material is always represented by empty spaces owing to its ready solubility in the acetone used for dehydration (Fig. 14); such spaces are not evident in permanganate fixed material but do contain a floculent material. In many cases this large, membrane-limited carbohydrate is one-half the volume.

Muciferous bodies

The only additional information to add about these bodies is first that they are very numerous. A section displaying as many as appear in Fig. 28 is typical. Second, though their microanatomy has not been completely worked out, it is clear that each organelle is effectively a small-walled compartment with semi-opaque contents (Fig. 28).

Contractile vacuole

The contractile vacuole occupies a characteristic position beside the nucleus (Fig. 17). The lumen of the vacuole is limited by a somewhat convoluted bounding membrane. No completely collapsed vacuole was observed.

Cytoplasmic organelles

The most conspicuous of the cell contents are the vast array of vesicles which crowd the cytoplasm between the other organelles. Most of the vesicles which are apparently empty, are especially conspicuous near the outside of the cell (Figs. 9 and 12). They are probably of more than one kind, but though none are yet chemically indentifiable, they resemble superficially the cytoplasmic vesicles commonly encountered in other cells of lower plants.

Cell division

One cell was observed in late division (Fig. 29). The nuclei with their nucleoli reorganizing are visible. Cell cleavage is evident on the surface of one side. The single Golgi body marks the vicinity of one of the flagellar poles. It appears that nuclear division is the last act of cell division before the cell cleaves. Quite conspicuous is the absence of scales which are found on a non-dividing cell.

Flagella

Chrysophaerella is placed in the family Mallomonadaceae (Smith, 1950; Fritsch, 1935) because of the described presence of a single flagellum. Figures 26 and 28 show non-dividing cells with a cross section and a longitudinal section of two flagella. With the light microscope, the second flagellum was noted by Korschikov (1941) and corroborated by Skuja (1948), but in all recent classification

schemes these reports have been overlooked or ignored. By careful observation, the writer also has been able to observe two flagella both under phase-illumination and light microscopy of living cells.

Korschikov (1941) placed Chrysophaerella in the family Synuraceae based on the presence of scales and the second flagellum. Manton (1955a), working on Synura did not observe circular profiles separating the chromatophore and nucleus. Wujek (unpublished observations) also has failed to observe such tubules in Synura petersenii or in Mallomonas caudata (Mallomonadaceae). Thus, on the basis of the second flagellum and the presence of the circular profiles between the chromatophore and the nucleus as observed in all members of the Ochromonadaceae to date, including this study, the writer places the genus in the family Ochromonadaceae.

Dinobryon Ehrenberg, 1833

Dinobryon sertularia (Fig. 30). was the first described and is designated the type species for the genus. Kreiger (1930), Ahlstrom (1937) and Fott (1959) have noted its cosmopolitan distribution. Kreiger (1930) incorporated into D. sertularia the species Dinobryon thyrsoideum Chodat (1897), Dinobryon sertularia var. thyrsoideum (Chodat) Lemmermann (1900) and also Dinobryon protuberans Lemmermann (1899a). Mack (1951) enlarged the species with the variety vindobonensis.

The cells of D. sertularia lie within loricas which may occur singularly or in compact colonies of 2-40 cells, dichotomously or rarely trichotomously branched. A lorica is 25-40 μ long, 10-12 μ wide with a flared aperture 12-15 μ in diameter. In outline the lorica approximates a short cone with a swollen central portion, a slightly campanulate aperture and a bluntly pointed base. The lorica is somewhat irregular in shape in the basal portion.

The ovoid to spindle-shaped protoplast which is attached to the lorica by means of a short, basal contractile filament, contains one or two parietal golden-brown chromatophores with a stigma. Two contractile vacuoles occur sub apically or are medially. The ochromonad type of flagellar apparatus (Bourrelly, 1961) is inserted on an oblique surface. The longer flagellum is approximately equal to the cell length. The storage products include small, scattered fat droplets and a basal chrysolaminarin mass.

The plate-like chromatophores, which are without pyrenoids, are parietal and slightly spiraled. They fill or occupy half to three-quarters of the circumference. Freqently the chromatophores are bi lobed with a narrow isthmus connecting the two segments. The single nucleus is in a median position. Meyer (1965) has observed up to five golgi bodies in a single cell.

Dinobryon sertularia has been observed to ingest bacteria and other particles. These particles are carried to the

protoplast by the movement of the long flagellum. After the flagellum strikes the protoplast, the cytoplasm flows around it to form a food vacuole. Undigested materials are discharged from the cytoplasm at any place in the upper half of the protoplast.

The composition of the lorica is uncertain. The life history of D. sertularia has been worked out by Meyer (1965).

Electron microscopy:

Chromatophore

The only knowledge of fine structure of this genus is the work of Joyon (1963) on Dinobryon divergens. The present study has revealed some features not noted before which are in contradiction to the observations of Joyon.

The most striking feature of the chromatophore is the anastimosing lamellae (Figs. 31 and 32). Joyon (1963) made no reference to this striking characteristic. As in other Chrysophyceae, the lamellae are four banded with the two inner bands thicker than the two outer bands (Fig. 34). The lamellae are stacked in rows of 6-12 in each chromatophore. As has been observed in other Chrysophytes of this study, the outer nuclear envelope is continuous with the outer chromatophore envelope. In addition, each chromatophore has two membranes proper.

Eyespot

The eyespot consists of several layers of closely packed granules situated at the anterior end of the plastid and adjacent to the whip-lash flagellum base. Great variation from cell to cell is found in the degree to which the pigment is retained after fixation. When fully retained the pigment appears intensely black (Fig. 33) even when the section is unstained. This is probably the truest picture, but the exact physiological or chemical reason why certain groups of cells behave like this while others in the same block do not is difficult to ascertain. Unlike the stigma of Euglena gracilis, which is found outside the plastid (Gibbs, 1960), the stigma of D. bertularia is found within the plastid membranes.

Nucleus

This structure is comparable to that of Hydrurus foetidus (Joyon, 1960, 1963, 1964). The center is occupied by a voluminous nucleolus (Fig. 31). It can also be seen (Figs. 32 and 34) that the nucleus is separated from the chromatophore by a narrow space which contains a number of circular profiles. These circular profiles represent sections of membrane-limited tubules which measure up to 0.45μ in longitudinal section. Joyon (1963) did not observe the presence of these tubules in Dinobryon divergens. In Figs. 35 and 36 are cross sections of D. divergens and D. bavaricum

also revealing the presence of these structures.

The outer membrane of the nuclear envelope outfolds to form a triple-membraned chromatophore envelope (Figs. 37 and 42). It appears, therefore, that the entire chromatophore is enclosed within a triple-membraned sac, one membrane of which is the outer envelope proper.

Mitochondria

Mitochondria are ovoid to slightly elongate, again with the characteristic double limiting membrane. The randomly occurring mitochondria numbered no more than seven in any one section. Meyer (1965) observed up to six with phase-contrast illumination.

Golgi

Meyer (1965) observed up to five Golgi bodies in his study of D. sertularia. Limited serial sections revealed only up to two Golgi bodies in any one cell (Figs. 38 and 39). They did not take on the appearance of being perinuclear as is true of other Chrysophyceae observed to date. Stacked cisternae numbered 6-12. They were surrounded by many small vesicles detached from the edges of the cisternae of origin.

Food reserves

The chrysolaminarin mass is located at the base of each cell (Fig. 40). It occupies from one-fourth to one-half of the cell volume.

Scattered about the cell are numerous oil droplets 0.5-5 μ in diameter. Many of the larger droplets have mitochondria surrounding them or attached to the surface of the droplet itself (Fig. 41).

Because Dinobryon can ingest food, many vacuoles are filled with food particles (Figs. 42 and 43).

Contractile vacuoles

Within the protoplast are one or more contractile vacuoles (apical, median or basal in position). Figure 40 is an oblique section showing a median contractile vacuole partially distended.

Muciferous bodies

Very small refringent bodies (light microscopy), possibly corresponding to the "muciferous bodies" of some workers (Chadefaud, 1935, 1947; Joyon 1963) occur in the peripheral cytoplasm. One of the species studies (D. sertularia) was examined for these bodies in the light microscope by Meyer (1965). He showed that they were not present in material from axenic culture; however, if bacteria were added to the cultures, the bodies were found. Thus it would seem to indicate that the "corps muciferes" of Chadefaud are nutrient artifacts. A review of the features of vacuoles is discussed in Bourrelly (1957). Several muciferous bodies are illustrated in Figs. 34 and 39.

Other cytoplasmic structures

There is a limited amount of endoplasmic reticulum underlying the cell membrane. It may be seen in both longitudinal and cross section (Figs. 44 and 45).

Statospore

Statopsores are quite frequent in populations of D. sertularia (Fig. 46). They differ from cysts of other genera in that they are binucleate (Geitler, 1935). While no observations on cyst formation are presented, several sections contained cyst material. Due to the nature of the cyst wall, fixation was not satisfactory. However, several observations may still be made. The apparent reduction in number or complete absence of mitochondria (Figs. 47 and 48) is quite striking. A much larger accumulation of oil droplets are present then is otherwise present in a non-encysted protoplast.

The chromatophore is much smaller and the tubules associated with it are not evident. Unfortunately, no evidence is present confirming the presence of two nuclei. The chrysolaminarin granule, while quite conspicuous in a vegetative cell, is quite reduced. No Golgi bodie were observed.

Lorica

While no attempt has yet been made to trace the mitotic stages as such, significant information regarding the mode

of production of the new lorica around each daughter-cell was obtainable.

In the formation of the lorica, a small funnel-shaped piece first arises at the base and thereupon, the protoplast rotates on its axis, following a spiral course and slowly secretes the remainder of the envelope (Klebs, 1893; Kreiger, 1930). When the latter is complete the protoplast withdraws to the base.

The lorica is probably formed from the many small particles liberated from vesicles derived from the Golgi body (Figs. 49 and 50). The profuse development of cisternae and vesicles crowed with small particles can be recognized as characteristic of a cell in predivision condition. Coalescence of the particles to form the lorica, which is thus similar to theca development in Platymonas (Manton and Parke, 1965), was not observed. These particles, and hence the lorica derived from them, are thus homologous with scales.

Electron microscope observations of the lorica of D. sertularia indicates that the lorica is constructed of a series of fine fibrils (Fig. 51), but their nature would seem to indicate that they are neither of cellulose nor hemicellulose, though their chemical composition was not determined. In most species the lorica is composed of a single layer, but in some species iron and manganese salts cover it. Bourrelly (1957) considers such species to have a lorica composed of two layers.

Epipyxis Ehrenberg, 1838

Epipyxis is described as being solitary, sedentary and with a smooth lorica. Later, the genus Hyalobryon was established by Lauterborn (1896) on the basis of a single species, Hyalobryon ramosum. This species was characterized by the close resemblence of its protoplast to that of Dinobryon and Epipyxis; by its bush-like colonies which differ from Dinobryon in that the younger individuals are attached to the outside of the mother koricas; and, by having extremely thin, translucent loricas composed of funnel-form growth rings fitted into one another, giving the margin of the lorica a denticulate appearence in optical section.

In 1963, Hilliard and Asmund reduced Hyalobryon to synonomy with the genus Epipyxis. Their reduction was based on observations that the described species of Hyalobryon and Epipyxis have loricas composed of scales.

The shape of the lorica is the taxonomic character at the species level. Morphologically, the loricas may appear tubular, cylindrical, vase-shaped, cup-shaped, spindle-shaped or campanulate. They may have a flaring or a somewhat constricted apex with a rounded or pointed base. The lorica wall is usually hyaline.

The outline of the scale varies among the species. The margins of the scales overlap one another. Size, shape and arrangement may vary from top to bottom of the lorica and these features are of diagnostic value.

The protoplast bears considerable resemblence to that of Dinobryon. It is irregularly spindle-shaped, tapering at the base to a delicate stalk by which it is attached to the base of the lorica wall. Except for the basal stalk, the protoplast is not in contact with the lorica wall. The protoplast is capable of moving briskly and is often abruptly withdrawn by the contractile stalk and then slowly resumes its extended position. These actions may be attributed to its heterotrophic means of gathering food (Hilliard and Asmund, 1963) or to irritability.

In the protoplast there is ususally one parietal, band-shaped chromatophore, the axis of which follows the protoplast of the cell in an obliquely ascending curve. When two chromatophores are observed, it may be due to the inconspicuousness of the middle part of the single chromatophore or the chromatophore may be divided preliminary to division of the protoplast (Lund, 1953; Skuja, 1948). The occurrence of one or two chromatophores is thus of doubtful taxonomic value.

Presence of a stigma is in question. Presence or absence of the structure has been used as an important character (Bourrelly, 1957), but it is of questionable value. In live specimens, Hilliard and Asmund (1963) have observed individuals with and without a stigma. Skuja (1956) suggests that young protoplasts possess a stigma which gradually disappears as they become older.

Contractile vacuoles may be situated in an apical, median or basal position. A chrysolaminarin mass of variable size is fraguently found in the posterior part of the protoplast. Oil droplets are also sometimes present. Two flagella of unequal length emerge from the apex of the protoplast, the longer one is a bi-seriate flimmer flagellum and the smaller a whip-lash flagellum (Petersen and Hansen, 1958).

Cyst formation has been observed. Lund (1953) records considerable variation in shape of the cyst in one species and suggests that the shape is of taxonomic value within limits. The morphology of the cyst is typical of that found among other Chrysophytes, possessing a plug with or without a collar.

Description of new species:

Epipyxis andrewsii sp. nov.: lorica 42-70 μ long., 4.5-9 μ lat.; in preparatione per "cresyl blue" tincta lorica ex ordinibus multis transversis squamarum irregularium composita videtur. Squamae discretae non observatae, itaque magnitudo formaque ignotae. Cellulae singulae aut penicillatim aggregatae; flagellum longum aeque long. et flagellum brevius ½ long. ac protoplastus; granulum leucosini 1/3-1/2 magnitud. cellulae; stigma nullum.

Cystes: duae observatae, ovatae ad subcordates; extremitas porum ferens as os loricae directa; obturaculum collareque nulla, apertura, autem, tholo

plasmatis magno tecta; systis a solo loricae usque ad 2/3 spatii infra os sita; maior diametros cystis (9-12 μ long. ad 7-10μ lat.) membranan loricae ultra dimensiones normales extendere efficit.

Nomen huius speciee clar. Mr. Hollings T. Andrews honorat.

Epipyxis andrewsii sp. nov.: dimensions of the lorica 42-70 μ long, 4.5-9 μ wide; in brilliant cresyl blue stained preparation, the lorica is composed of many transverse rows of irregular scales; no scales were observed free to determine dimensions and shape; cells solitary or in tufted groups; long flagellum equal to the length of the protoplast and the shorter flagellum one-half as long; leucosin granule one-third to one-half cell size; no eyespot present.

Cysts. Only two were observed: oval to subcordate; pore-end directed toward mouth of the lorica; no plug or collar present, but opening covered with a large plasma dome; cyst lodged from the bottom of the lorica to a distance two-thirds from the mouth; greater diameter of cysts (9-12 μ lond to 7-10 μ wide) causes lorica wall to expand beyond normal dimensions.

Species named in honor of Hollings T. Andrews.

Electron microscopy:

Repeated attempts using standard and modified fixation

techniques did not result in a satisfactory preparation. Lack of readily available material did not allow a full spectrum of fixation routines to be carried out. Thus, only a limited amount of information concerning the fine structure of Epipyxis andrewsii (Figs. 52 and 53), can be made at this time.

Lorica

As has been noted in light microscopy, the lorica is composed of scales. Figure 54 illustrates the scale overlap and that the lorica may contain as few as four or as many as eight scales in cross section.

Chromatophore

In E. andrewsii the lamellae are arranged in bands of four with the inner two bands double the thickness of the outer two (Fig. 55). The inner bands can be seen to be made up of two thin bands that are in reality only closely appressed. Scattered among the lamellae are oil droplets. No pyrenoid could be observed.

Nucleus

The best single view of the nucleus is in Fig. 56, though parts of it appear in other sections. The nucleus is pressed closely against the plastid and follows the configuration of its surface. No tubules could be observed separating the nucleus from the chromatophore, but this is thought to be the fault of fixation much as Joyon (1963) experienced in D. divergens.

Golgi

As far as could be determined, there is only one Golgi body present (Figs. 55 and 57). As in Dinobryon, this Golgi also is not perinuclear. Four to eight cisternae are present.

Mitochondria

Mitochondria are very well shown in this material and they may be picked out in a number of sections. No more than two were observed in any one section.

Other components of the cytoplasm

Vesicles of various kinds make up most of the other components of the cytoplasm. There are a few laterally placed vesicles bounded by membranes, but with no very obvious contents (Fig. 55).

There are several large to small lipid bodies in the cytoplasm (Figs. 58 and 59). They may be very close to the surface of the cell or more deeply seated. Sometimes thay may be within a vesicular membrane, at other times this is less obvious.

Food vacuoles are present in some of the sections (Fig. 60). Most of the rest of the cytoplasm is occupied by vesicles of intermediate size and with greater or lesser amounts of granular contents suggesting chemical precipitation of tenuous material.

Ochromonas Wystozki, 1887

Ochromonas cells are solitary, free-swimming and contain

one or two laminate chromatophores. Sometimes a pyrenoid is present (Bourrelly, 1957). An eyespot may oy may not be present. There are two flagella of unequal length, one a flimmer and the shorter, a whip-lash.

Contractile vacuoles are located toward the anterior end. A large chrysolaminarin mass occupies the posterior half of the cell. Also, scattered about the cell are numerous highly refractive oil droplets.

Statospores with conspicuous plugs are produced endogenously (Doflein, 1922). Spines may or may not be present on the cysts. Numerous species have been described only on the vegetative cell and without the observation of the cysts. This has undoubtedly filled the literature with many species which are not adequately described without knowledge of the cyst morphology.

Description of new species:

Ochromonas thompsonii sp. nov. (Figs. 61 and 62): cellulae sphericae chordatae ovatae pyriformesque, fusiformes ad ellipsoideas; 5-15 μ long., 5-11 μ lat.; chloroplastus unus, flavo-brunneus, lobatus, sine pyrenoideo; granulum leucosini magnum, usque ad dimidium magnitudinis cellulae; guttulae olei multae; flagellum breve dimidio brevius quam flagellum longum; stigma nullum.

Cystes globosae ad ovatas; 10-12 μ diam.; porus

collari externo elvato praeditus; membrana levis.

Typus: In collectione culturarum algarum vivarum (Wujek) Univ. Kansas. Nomen huius speciei clar. Dr. Rufus H. Thompson.

Ochromonas thompsonii sp. nov. (Figs. 61 and 62): cells spherical, chordate, ovoid, pyriform, fusiform to ellipsoid; 5-15 μ long by 5-11 μ wide; one yellow-brown lobed chloroplast lacking a pyrenoid; large, up to one-half cell size, leucosin granule; many oil droplets; short flagellum one-fourth length of long flagellum; no eyespot present.

Cysts globose to oval; 10-12 μ in diameter; pore raised external collar; smooth wall.

Type: in living algae culture collection (Wujek) University of Kansas. Species named in honor of Dr. Rufus H. Thompson.

Electron microscopy:

Chromatophore

The ultrastructure of the chromatophore is essentially similar to that of Ochromonas danica (Gibbs, 1962b) in that the plastid is characterized by being enclosed within a triple membrane, the outer membrane being an outfolding of the nuclear envelope. This outer envelope is best illustrated here in Fig. 63. It can also be seen clearly in Fig. 66 that the chromatophore envelope is characteristically

much thicker than the nuclear envelope.

The narrow space which separates the chromatophore from the nucleus contains a number of tubules 250 to 350 A in diameter (Fig. 64), but in places they are absent. These circular profiles (Gibbs, 1962a, 1962b) represent sections of membrane-limited tubules and have been seen in longitudinal sections to extend up to 0.5 μ. In some sections, tubules are not present in the space between the nuclear envelope and the chromatophore and here the distance between the two is less than 100A.

The plastid lamellations are made up of four bands (Fig. 71) as in other Chrysophytes examined in this study. The lamellae may be interconnected, but true grana are not formed. No pyrenoid or eyespot is visible in any of the cells.

Golgi

The single Golgi body is situated beside the flagellar bases and near to the nucleus (Fig. 71). It is usually surrounded by many small vesicles detached from the edges of its own cisternae (Fig. 70).

Nucleus

Ochromonas thompsonii has a nucleus averaging 2.5 μ in diameter. The nucleolus is very small and usually situated toward the posterior end of the nucleus (Fig. 69).

Attention should be drawn to the part of the nuclear

surface included in Fig. 63 and to the nucleus in Fig. 68 to substantiate the statement that in this, as in other cells in this study, the interior of the nucleus is separated from the body of the cytoplasm not only by membranes, but also by a translucent, apparently liquid layer which in any one section appears to be discontinuous, but which could equally by a single circum-nuclear vesicle (Palade, and Porter, 1954) or perinuclear space bridged at intervals.

Mitochondria

The mitochondria range from small, bacilliform to straight or curved organelles. They lie along the chromatophore surface near to the nucleus. The cristae are small, swollen finger like and are densely crowed.

Oil droplets

Located about the periphery of the cell are large oil bodies. They are frequent (4-27) in both cultured and field material and are considered to be an accumulation of food reserve that diminishes if the organisms are kept in the dark for extended lengths of time (Smith, 1955). Their characteristically dense, amorphous appearance (Fig. 67) in electron micrographs is probably sufficient evidence that they are not comparable to the leucosin bodies in other algae (Manton, 1961; Gibbs, 1962c). The location of oil bodies may be predetermined by the location of special areas in the cytoplasm that become inflated during active periods of photosynthesis.

Light microscope studies of O. thompsonii indicate that the oil bodies shown in Figs. 64 and 67 are typically located.

The oil droplets in the chromatophore (Fig. 64) appear to have the same density as the larger oil droplets, but the compositional identity of the two types of structures are unknown.

Contractile vacuole

As described by Lang (1963) in the Volvocaceae and Astrephomenaceae, the contractile vacuole is delimited by a single membrane and when fully distended, this layer is quite obvious (Fig. 66). When the contractile vacuole is completely collapsed (Fig. 65), the single membrane is folded radially.

Muciferous bodies

The vesicles of slightly opaque material visible between the chrysolaminarin and plasma membrane in Figs. 68 and 72 are the muciferous bodies. As surmised from their behavior when seen discharging under the light microscope, these are not merely amorphous drops or granules of material, but organelles with a definite microanatomy. This has not been completely worked out, but from the figures it is clear each organelle is effectively a thin walled compartment with semi-opaque contents. There are signs of further subdivision of the contents, notably in the faint white internal contours in the central body.

Chrysolaminarin

Figure 68 is an oblique section through a cell showing the large chrysolaminarin mass. This vacuole is limited by a single membrane and is the site of carbohydrate storage. In stale cultures, the leucosin is replaced with a dense, heterogeneous substance (Fig. 73), similar to the smaller oil droplets in actively growing cultures. The exact nature of the body could not be determined.

Food vacuoles

Food vacuoles are distinguishable from other cytoplasmic vesicles by their irregular contents. Several food vacuoles with rather indefinite contents are represented in Figs. 74 to 76.

Finally, there are the cytoplasmic vesicles (Fig. 68) most of which are apparently empty, though doubtless not so in life. They crowd the cytoplasm between the other organelles and are especially conspicuous near the ourside of the cell. They are probably of more than one kind, though none are yet chemically defined.

Chromatophore formation

While complete chromatophore development was not observed, several cells showed representative stages of development similar to that found in another Ochromonas species examined by Gibbs (1962b). The two large proplastids on either side of the nucleus (Fig. 63) are actually the two arms of a

single U-shaped proplastid which partly encircles the nucleus.

Uroglena Ehrenberg, 1833

Uroglena is a free-swimming colonial organism in which the individual cells have the morphology of an Ochromonas cell (Fig. 77). The individual Uroglena cells are ovoid to ellipsoid with two heterodynamic flagella inserted anteriorly. Petersen (1918) demostrated that the longer flagellum is the flimmer and the shorter whip-like. Adjacent to the shorter flagellum is the stigma (Conrad, 1938).

Within a cell is one large parietal chromatophore. Some cells contain two chromatophores, but it is not clearly established that this is characteristic of the vegetative cell. It may well be that these cells are about to undergo division.

One or more contractile vacuoles are present in the anterior half of the cell. Posteriorly oriented is a dull appearing mass referred to as leucosin or chrysolaminarin. Highly refractive oil globules are scattered about the cell. The nucleus is central in position. Cell division is longitudinal. Daughter colonies are formed by fragmentation or constriction of the parent colony.

The cells are joined by radiating, dichotomously branched system within the colony. Bourrelly (1957) has termed them mucilage threads, possibly because they stain easily with brilliant cresyl blue or methylene blue. Osmic acid fumes also stain these threads black, suggesting that they are

protoplasmic (Fig. 78).

Uroglena forms statospores endogenously as is typical of other Chrysophyceae. The cyst wall, smooth or ornamented with spines, is composed of silica. In addition there may be a wide collar concentric with the pore and its mucilage plug.

Critical observations of the flagella indicate that the whip-lash (10-20 μ) is approximately one-half the length of the flimmer (30-40 μ) and at least as long as the cell body. The flimmer flagellum is characterized by having biseriate mastigonemes originating from bands (Fig. 79).

Uroglenopsis Lemmermann, 1899b

Uroglenopsis is a colonial planktonic chrysophyte. Originally it was described by Calkins (1892) as Uroglena americana but later was made the type of the new genus by Lemmermann. A Uroglenopsis cell has the morphology of a Uroglena cell.

Troitzkaja (1924) later referred Uroglenopsis back to Uroglena. Most authors have followed this treatment to date. Thompson (unpublished) recently has shown characters which may validly be used in separating these genera. These are based on the following criteria: the whip-lash flagellum of Uroglenopsis is less than one-half the length of the flimmer, while in Uroglena, the whip-lash flagellum is at least one-half or more the length of the flimmer. Also, the strands of the branched system are broad in Uroglenopsis

(Fig. 80), but are thread-like in Uroglena (Fig. 78).

Electron microscopy:

As no consistent differences in the fine structure were observed between the two genera, no distinction between them will be made in the following discussion. Also, because cysts delimit species, only two species can be identified in this study, Uroglenopsis notabilis Mack (Fig. 81) and Uroglena collaris Thompson (in edition, Fig. 82).

External morphology

Shadowcast whole mounts examined with the electron microscope did not reveal any scales. They did substantiate many observations from light microscope, i.e., relation of flimmer to whip-lash flagellum length (Figs. 83-Uroglena and 84-Uroglenopsis), as a generic character as proposed by Thompson. Also, the mastigonemes are biseriate and not uniseriate. The site of attachment of mastigonemes to the flagellum has not been determined with certainty. They are too fine to be followed in most sectioned material. However, the weight of evidence has led Manton (1955b, 1959) to conclude that they arise from two specific fibrils of the peripheral axis.

Chromatophore

In a previous investigation, Gibbs (1960, 1962b) showed that the chromatophore of Euglena and Ochromonas are limited by a triple membrane. The present investigation also reveals a triple membrane around the chromatophores of Uroglena

and Uroglenopsis. The micrograph used to demostrate the chromatophore is a cell fixed in permanganate. Figure 85 is a section through a plastid and part of the adjacent nucleus. It can be seen that the nucleus is separated from the chromatophore by a narrow space which contains a number of circular profiles (Gibbs, 1962a, 1962b). These circular profiles represent sections of membrane-limited tubules which measure approximately 250 to 350 A in diameter and have been seen in longitudinal section to extend up to 0.5 μ. In some sections, tubules are not present in the space between the nucleus and chromatophore. Pores are present in the region of the nuclear envelope which borders on the cytoplasm but are absent in the region which borders adjacent to the chromatophore (Fig. 85).

Pyrenoid

The cells appear to lack pyrenoids when observed with the light microscope; however, an electron micrograph of a sectioned cell (Fig. 86) reveals that a pyrenoid is present on the inner surface of the chromatophore. The pyrenoid is easily distinguishable from the rest of the chromatophore by the greater density of its matrix material. Also, the pyrenoid tends to project slightly from the inner surface of the chromatophore. In an attempt to illucidate the pyrenoid in light microscopy, stains and phase-contrast microscopy were employed, but all attempts failed.

While pyrenoids are relatively widespread in the Chryso-

phyceae, this pyrenoid differs from other Chrysophyte pyrenoids studied to date. Figure 87 illustrates the pyrenoid types described to date. It can be observed that the pyrenoid of Uroglena and Uroglenopsis lacks the conspicuous lamellae which traverse or appear scattered over the surface of other Chrysophyte pyrenoids. A similar condition is observed in the green alga Scenedesmus (Bisalputra and Weier, 1964). Bisalputra and Weier believe this to be an advance type of pyrenoid.

The pyrenoid varies from slightly concave to a structure possessing one or two projections that protrude into the adjacent nuclear region (Figs. 88 and 89). These finger-like extensions are present in the larger, more mature appearing cells. When viewed in transverse section (Fig. 90), they are bounded by three membranes, one of which is the outer nuclear envelope.

Eyespot

Located in the anterior half of the cell within the chromatophore is the eyespot or stigma. The eyespot lies directly beneath the cytoplasmic membrane and chromatophore envelope and consists of plates of large electron-dense granules closely packed together. There is no alternating arrangement of the plates and chromatophore lamellae as observed in members of the Volcocales (Sager and Palade, 1957; Lang, 1963; Lembi and Lang, 1965). Longitudinal and cross sections

indicate that the stigma consists of one or two rows of granules just within the chromatophore membrane (Figs. 92 and 93). Other organisms have more than one layer (Lang, 1963; Sager and Palade, 1957). Whether the number of rows depends upon the size of the cell is unknown. Figure 91 substantiates the observation of Conrad (1938) that the whip-lash flagellum is adjacent to the stigma.

Mitochondria

Mitochondria are limited by the usual double membraned envelope. Characteristically, the invaginations of the inner membrane which form the cristae are tubular or villus-like rather than plate like (Figs. 85 and 93). The shape of the mitochondria is highly variable and there is no apparent association of them with other organelles aside from the flagellar bases. In general, the mitochondria tend to be along the margin of the cell.

Golgi

Immediately below the attachment of the flagellar bases is an area occupied by vesicles of the Golgi body. The Golgi area is commonly bounded by the nucleus, mitochondria and chromatophores (Fig. 94), though it is never separated from the flagellar bases by any of these organelles. It is not delimited by a membrane as in Tetracystis (Brown and Bold, 1964). When cut tangentially or merely grazed, only the compacted vesicles and traces of flattened membranes

visible (Figs. 98 and 99). When cut more nearly centrally, however, a surprising complexity of structure is revealed. Figures 94 and 95 are inserted to illustrate this.

Chrysolaminarin

Chrysolaminarin in sections is represented by the large empty space in the posterior half of the cell. The empty space is due to its ready solubility in the alcohols and acetones used for dehydration. Such spaces are very liable to mechanical distortion, but a demostratable undistorted cavity bounded by a membrane is contained in Fig. 88.

Oil droplets

The occurrence of oil droplets adjacent to the plastid (Fig. 96) supports the contention that the plastid may be envolved in the elaboration of the lipid material.

Contractile vacuole

The contractile vacuole is represented in sections by a large and apparently empty cavity. Figure 97 shows the contractile vacuole in its closed position.

Flagella

In Figs. 94 and 100 details concerning the attachment of the flagella are illustrated. The flagella are situated close to one edge of the plastid. Figure 100 shows the position of one of the roots in relation to the subtending basal body. There is no sign of direct attachment to the

nucleus. Connections between the flagellar bases and the cell surface or plastids are certainly more important mechanically than any direct association with the nucleus.

Food vacuoles

Phagotrophy is of a very common occurrence, the cells ingesting organisms up to about 3 μ in size (Fig. 101). The presence of more than one food vacuole was not unusual. The actual ingestion of material occurs near the flagellar poles. The whole process is followed by a breakup of the material (bacteria, etc.) into smaller granules (Fig. 102). Many other vacuoles are filled with indiscernable material (Fig. 103).

Cytoplasmic granules

Since extreme metabolic significance has been attributed to certain types of granules (Palade, 1956), it is important to notice their appearance here even though there are no chemical data to add. Granular cytoplasm fills the interstices between the membrane-limited vesicles and other cell organelles in all parts of the cell. The granules may be seen in Figs. 104 and 105.

Cell division

Knowledge of cell division by longitudinal division is known from light microscopy. While it was not possible to follow cell division completely through electron microscopy, several sections presented information pertaining to it.

Figure 106 is a section through the anterior end of a dividing cell showing the duplicated flagellar bases. They have already pulled apart.

In Fig. 107, the chromatophore has divided. It seems also clear that pyrenoids do not disappear upon initiation of cell division as is the case in Hydrodictyon (Klebs, 1891) or Volvox (Overton, 1889). Figures 108 and 109 show the chromatophores with associated pyrenoids after completion of division pulling apart.

The final step is nuclear division. As the nucleoli duplicate (Fig. 110), the nucleus starts to divide eventually resulting in the final separation of the nuclei (Fig. 112). Finally, the cells are held together by a small protoplasmic bridge (Fig. 113) which eventually breaks (Fig. 114) to free the two cells.

CONCLUSIONS

In this examination of the Chrysophyte algae from two families, a consistent and in some ways unique pattern of cell fine structure has been found. Chromatophores are a prominent feature of the cytoplasm and contain lamellae, usually of four uniformly separated bands (thylakoids if the terminology of Weier, et al. (1965) is to be followed). This is very different from other groups, viz. Euglenophyta (Gibbs, 1960); Phaeophyta (Bouck, 1965), Rhodophyta (Bouck, 1962; Berkaloff, 1962), where the lamellae are two to twelve-banded. Why such a configuration is so consistent in this group and none of the other algae can only remain unanswered for the present. Also, the chromatophores generally are longitudinally oriented, flanking the nucleus with the lamellae running parallel to their long axes. Additional work on other fresh-water Chrysophyceae would be helpful in establishing the extent of this uniformity and ultimately, what its relation is to the photosynthetic process.

The chromatophore envelope consists of three membranes and follow the contour of the pyrenoid if present. The outer chromatophore membrane is smooth and continuous with the outer membrane of the nucleus. The two inner membranes limit the chromatophore proper. On the surface of the plastid, the two inner membranes divide in the region of the nucleus to allow for the presence of tubules. The exact

nature of these tubules appear to be infoldings of the outer chromatophore membrane proper. These relationships are diagrammed in Fig. 115.

The relationship of the shorter flagellum, when present, with the eyespot as reported by Conrad (1938) in Uroglena holds true for the other genera in this study. No direct contact through fibrils, microtubules or extending vesicles are noted; however, the extreme closiness of the stigma and whip-lash flagellum favors some type of correlation.

The use of flagellar bases as phyletic indicators is only beginning (Manton, 1965). This is particularly true in the transition region between base and flagellum proper. In the green algae, the transition region contains a spectacular stellate pattern. A search for it among the Chrysophyceae studied in this investigation proved fruitless. It was found with difficulty in only one Chrysophyte, Prymnesium parvum (formerly Chrysophyceae, now Haptophyceae sensu Christensen, 1962).

In spite of the basic similarities among the taxa studied, there exist structural differences among them. The differences are the presence or absence of scales, the presence or absence of a pyrenoid and the number, length and type of flagella.

Observations on the spines and scales inside and outside the cell have given important insight into their probable mode of origin from internal vesicles and not from the ex-

ternal cell membrane. Evidence that the scales and spines are siliceous are given.

Some of the differences and similarities presently used in systematic arrangements of the Chrysophyceae are admittedly artifical. Papenfuss (1955) mentioned that some groups are separated on the basis of the number and relative length of the flagella. These characteristics are not of major importance, however, in the systematic arrangement in the other orders within the class. The establishment of flagellar characteristics as the most important criterion to be used in the systematics of the Chrysophyceae has been questioned by Korschikov (1929). In his account of Synura, Korschikov pointed out that the two flagella of the cells in this genus are not rqual and that they differ morphologically (this fact was first established by Petersen in 1918). In a later publication, Korschikov (1941) discovered that the cells of Chrysophaerella, previously described as having one long flagellum, also possessed a second, very short flagellum. Korschikov designated Chrysophaerella as a member of the Synuraceae on the basis of the similarity between the silica scales produced by Synura and Chrysophaerella.

To conform with the system of Korschikov would necessitate a complete revision of the systematics of the Chrysophyceae. Much more work on this group is needed before such a new viewpoint can be accurately obtained. Therefore,

for the present, it is best to follow the admittedly artificial systems based on flagellation. With future studies, however, cytological and fine structure features of the protoplast may assume a greater role in helping to clarify the taxonomic position and phylogenetic relationships among members of this group.

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KEY TO LABELING

All electron micrographs are of material fixed in OsO_4 unless otherwise stated.

B=Bacteria

b=bifurcation

C=Chromatophore

Chm=Chromatophore membrane

Cm=Cell membrane

Cv=Contractile vacuole

ER=Endoplasmic reticulum

Fb=Flagella base

Fv=Food vacuole

G=Golgi body

L=Leucosin(chrysolaminarin)

M=Mitochondria

Mb=Muciferous body

N=Nucleus

Ncl=Nucleolus

Ne=Nuclear envelope

Np=Nuclear pore

O=Oil droplet

P=Pyrenoid

r=Flagella root

S=Stigma(eyespot)

t=Tubules

Vc=Vesicular complex

Vm=Vesicular membrane

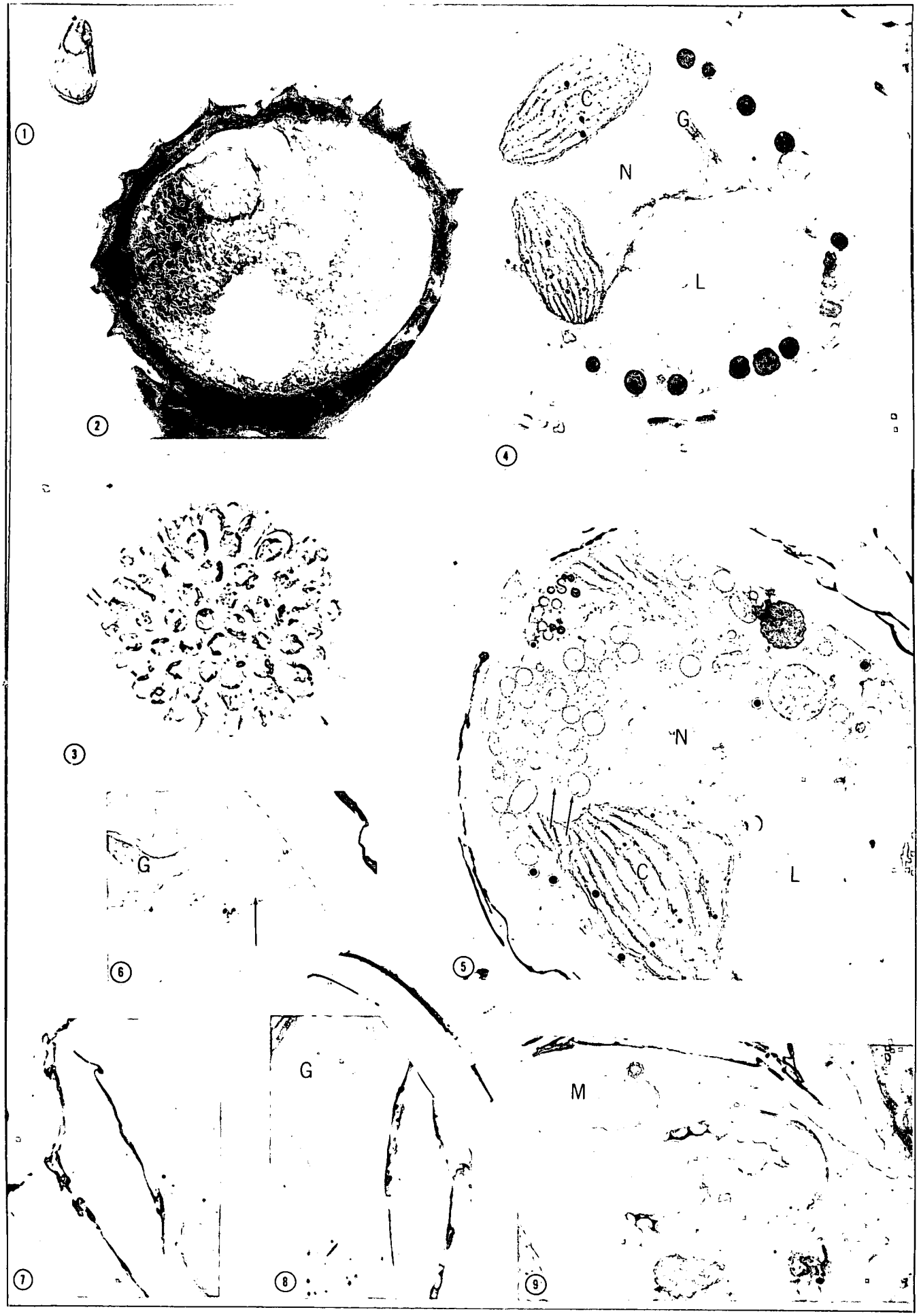
PLATE I.

Figures 1-2, Microglena punctifera Ehrenb.

1. Light micrograph. X 1,000.
2. Section of a cell to show the silica granules embedded in the periplast. X 31,000.

Figures 3-9, Chrysophaerella itascaei Thomp. and Wujek,

3. Light micrograph of a colony. X 1,500.
4. Cross section of a HF treated section showing the abundance of the normally appearing opaque scales. X 12,000.
5. Vesicles (arrows) that contain silica particles. Compare with Fig. 4. X 9,000.
6. Lamellation of the silica particles to form an internal scale (arrow). X 16,500.
7-8. Transfer of the scale to the outside of the cell. X 15,500.
9. Transverse oblique section through a partially completed scale. X 14,000.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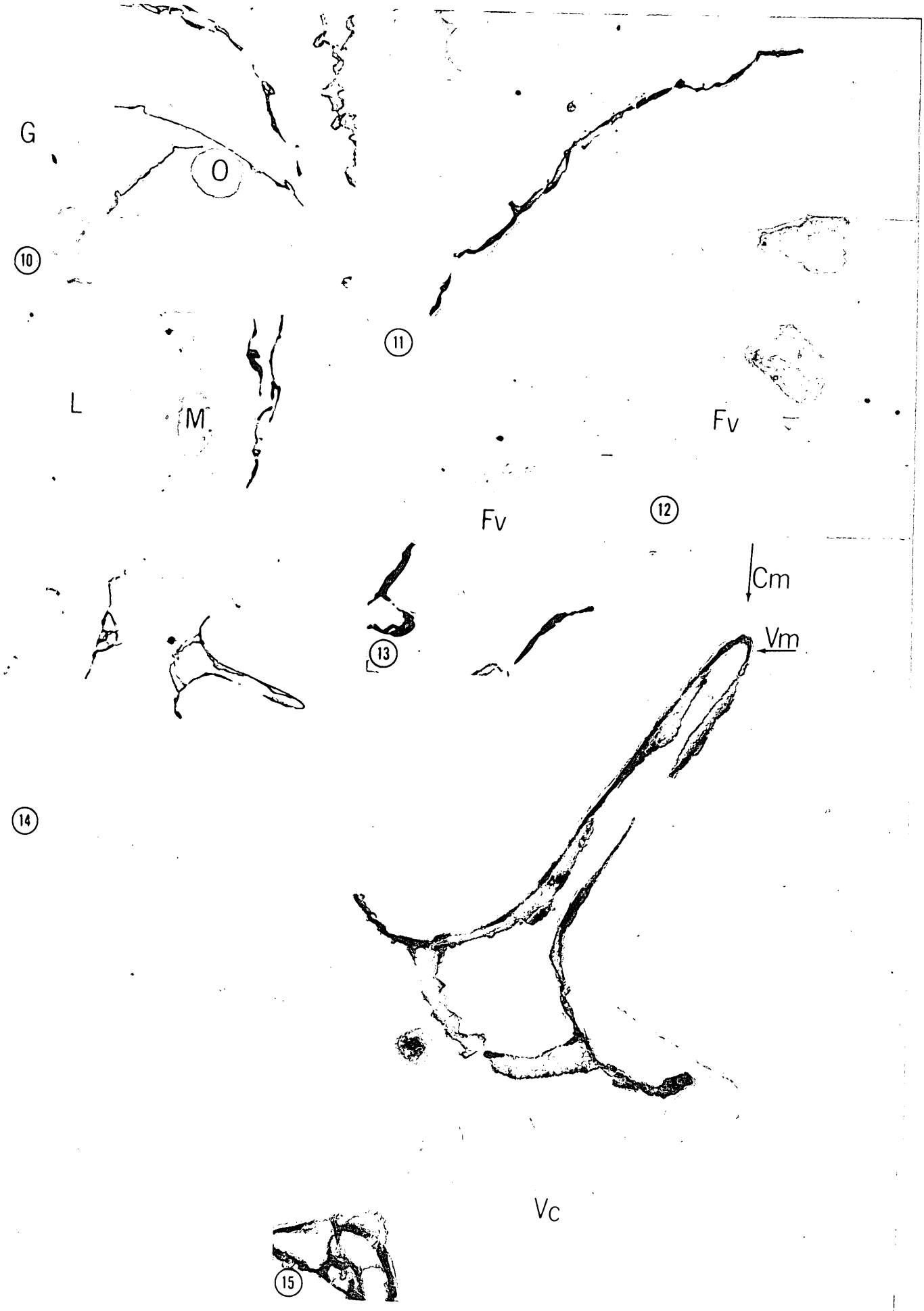
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PLATE II.

Figures 10-15, Chrysophaerella itascaei Thomp and Wujek.

10-11. Two sections through a group of vesicles bearing scales. X 14,000.

12-13. Transverse sections of two food vacuoles. X 18,000.

14. Part of a section showing an immerging spine. X 9,600.

15. More highly magnified part of Fig. 14 to show the membranes around the spine as it immerges from the cytoplasm. X 40,000.

PLATE III.

Figures 16-20, Chrysophaerella itascaei Thomp. and Wujek.

16. Potassium permanganate fixed section showing the three membranes (arrows) around the chromatophore. X 14,000.
17. A section through the anterior end of a cell to show the contractile vacuole. X 12,500.
18. General view of a cell showing the nucleus, Golgi body, plastids, oil droplets, besides vesicles and granular protoplasm. X 12,500.
19. Diagrammatic representation of the fine structure of the chromatophore lamellae. a. Splitting of a thick interior lamella of a band into two thin outer lamellae. b. Termination of a band of four appressed lamellae at the edge of the plastid.
20. Arrow indicates the terminations of four lamellae at the limiting membranes of the chromatophore. X 12,000.

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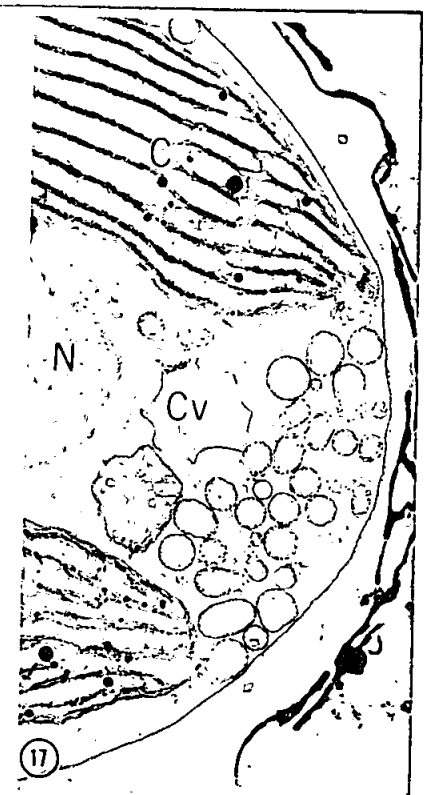

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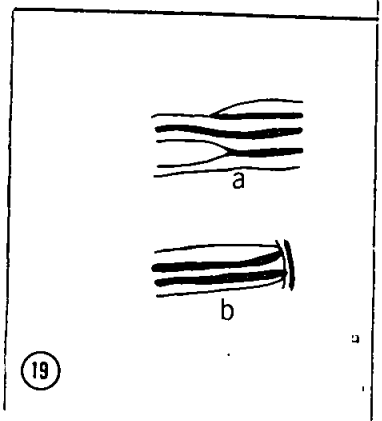

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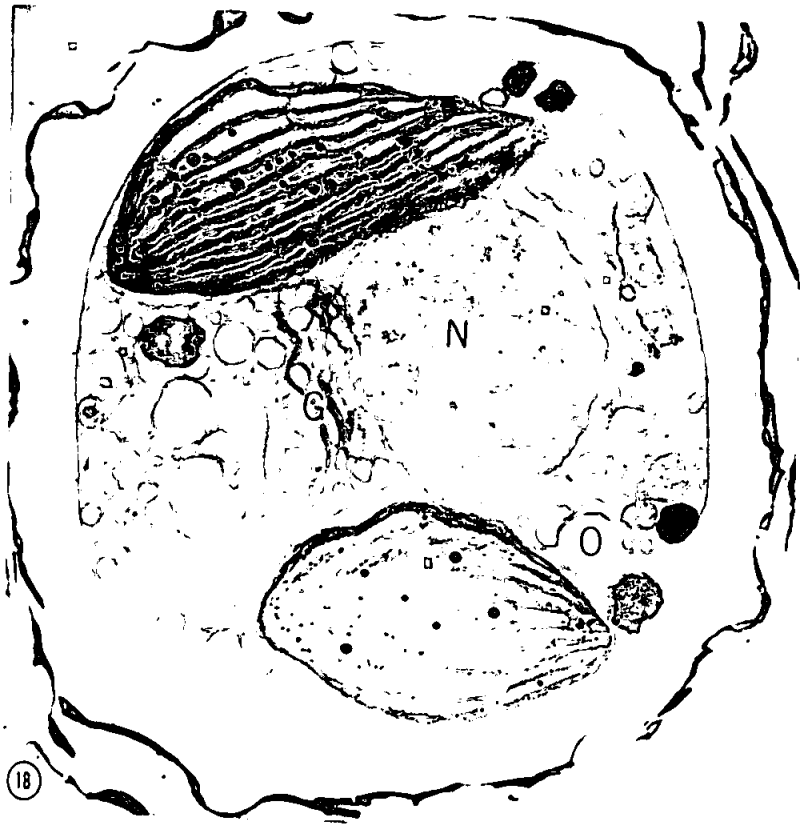

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PLATE IV.

Figures 21-26, Chrysophaerella itascaei Thomp. and Wujek.

21. Nucleus of C. itascaei. X 12,000.

22-23. Potassium permanganate fixed sections to show the outer membrane of the nuclear envelope outfolding to form the outer plastid envelope. X 22,000.

24. Cross section of the eyespot which lies within the cytoplasmic membrane and chromatophore envelopes. Muciferous bodies are present in the periphery of the cytoplasm. X 18,500.

25. Section through the basal region of the flagella showing a mitochondrion associated with them. X 16,000.

26. Section through a cell showing the flagella at approximately right angles to that of Fig. 25; the Golgi is associated at their base; muciferous bodies are scattered about the periphery of the cytoplasm. X 17,500.


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PLATE V.

Figures 27-29, Chrysophaerella itascaei Thomp. and Wujek.

27. Part of a section near the flagellar bases showing the cisternae of a Golgi body. X 14,100.

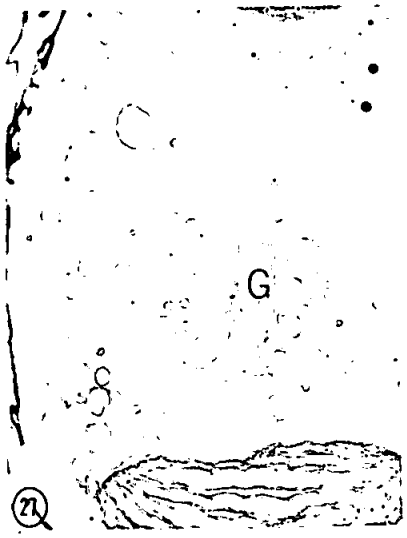
28. Transverse section of the flagella through the basal region. X 38,000.

29. Section through a cell in division; the absence of scales is striking. X 8,400.

Figures 30-31, Dinobryon sertularia Ehrenb.

30. Light micrograph of a colony. X 1,500.

31. Longitudinal section through the nuclear region; ribosomes (arrows) are present in the nucleolus, on the outer membrane of the nucleus and in the cytoplasm; a four banded lamellae is present. X 13,800.



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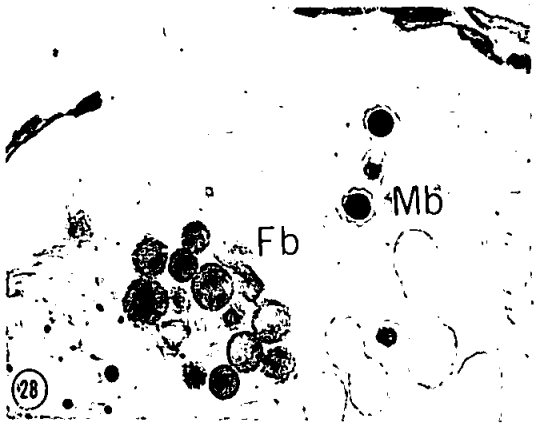

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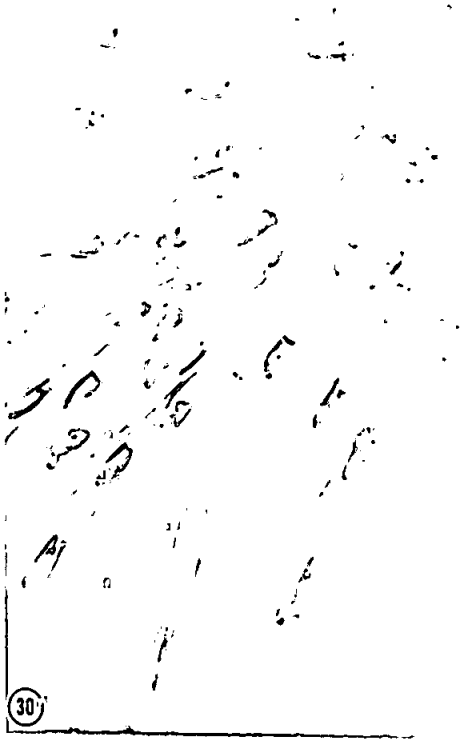

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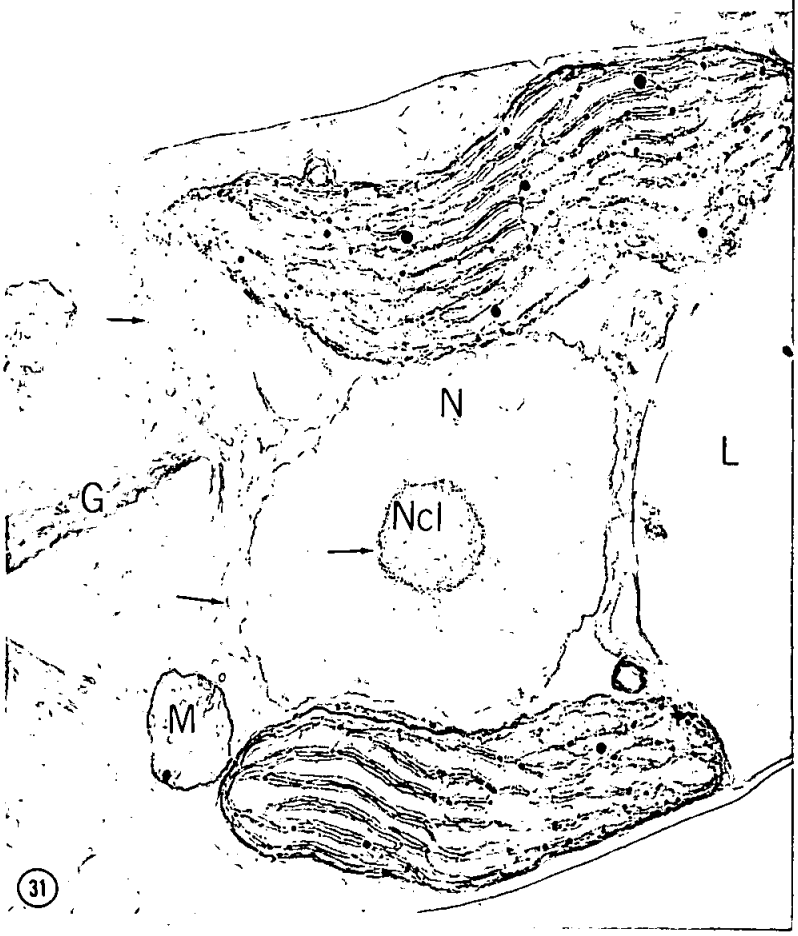

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PLATE VI.

Figures 32-34, Dinobryon sertularia Ehrenb.

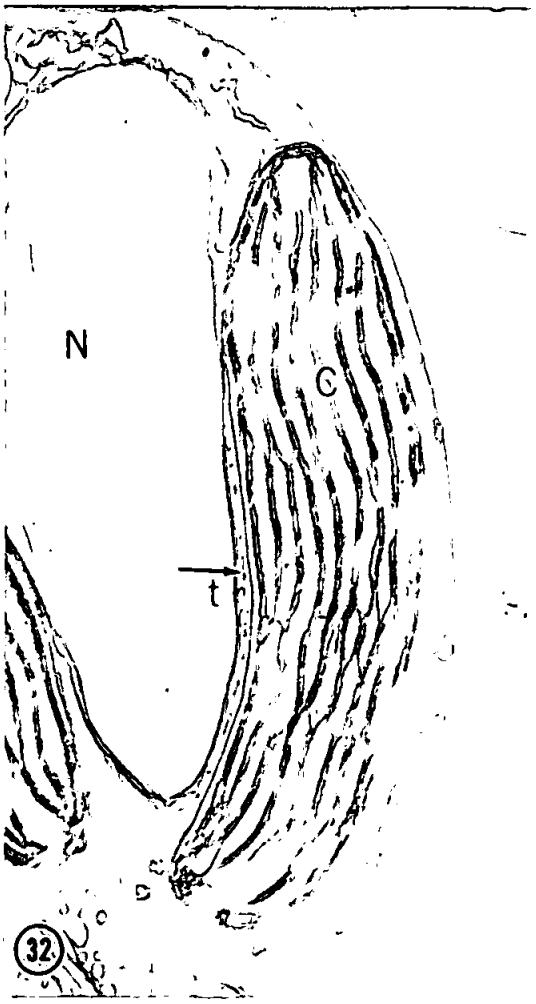
32. Part of a potassium permanganate fixed section showing the anastimosing lamellae; tubules (arrow) are present between the two membranes which delimit the plastid proper. X 20,500.

33. Part of a section through the anterior end of a plastid showing the eyespot within the limiting membranes of the plastid; also visible is a cross section of the whip-lash flagellum; the double thickness of the two inner lamellae bands is apparent. X 43,000.

34. Cross section through the nucleus ans chromatophore showing the presence of tubules (arrow) between them; a large muciferous body is present adjacent to the nucleus. X 23,200.

Figure 35, Dinobryon divergens Imhof.

35. A portion of a cross section showing the tubules (arrow). X 24,800.



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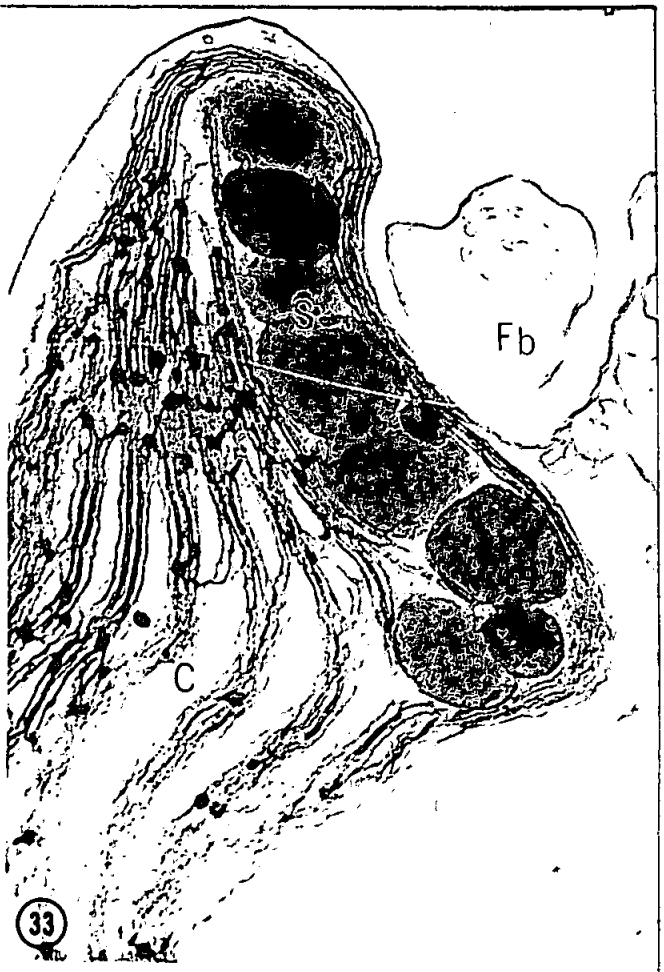

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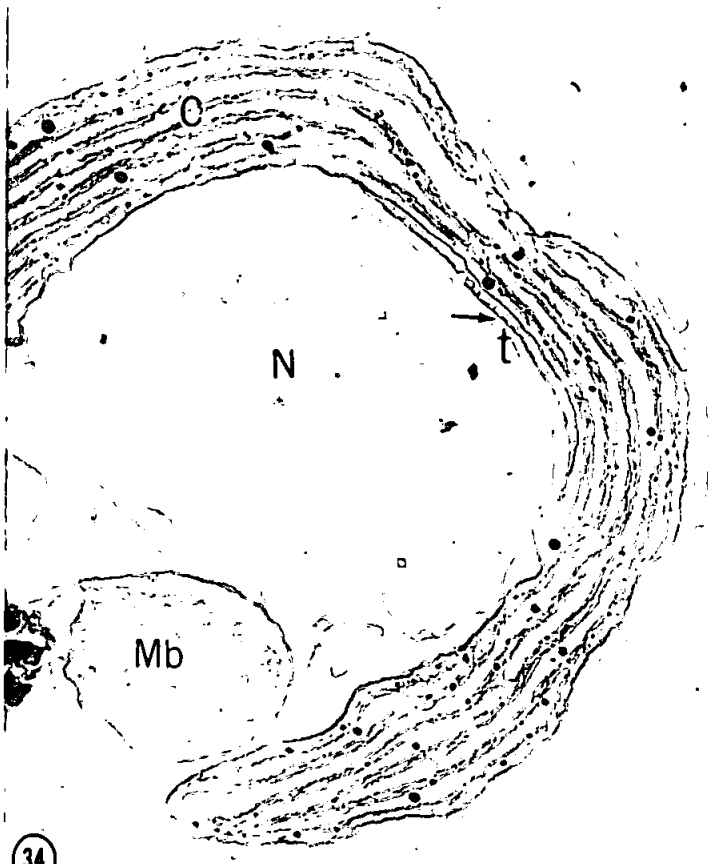

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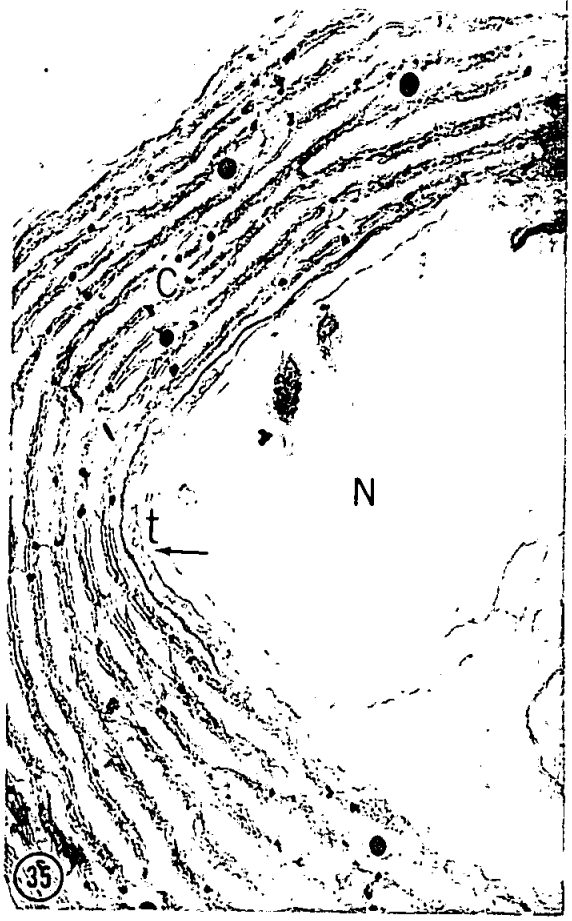

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PLATE VII

Figure 36, Dinobryon bavaricum Lemm.

36. Cross section showing the tubules (arrow). X 19,500.

Figures 37-42, Dinobryon sertularia Ehrenb.

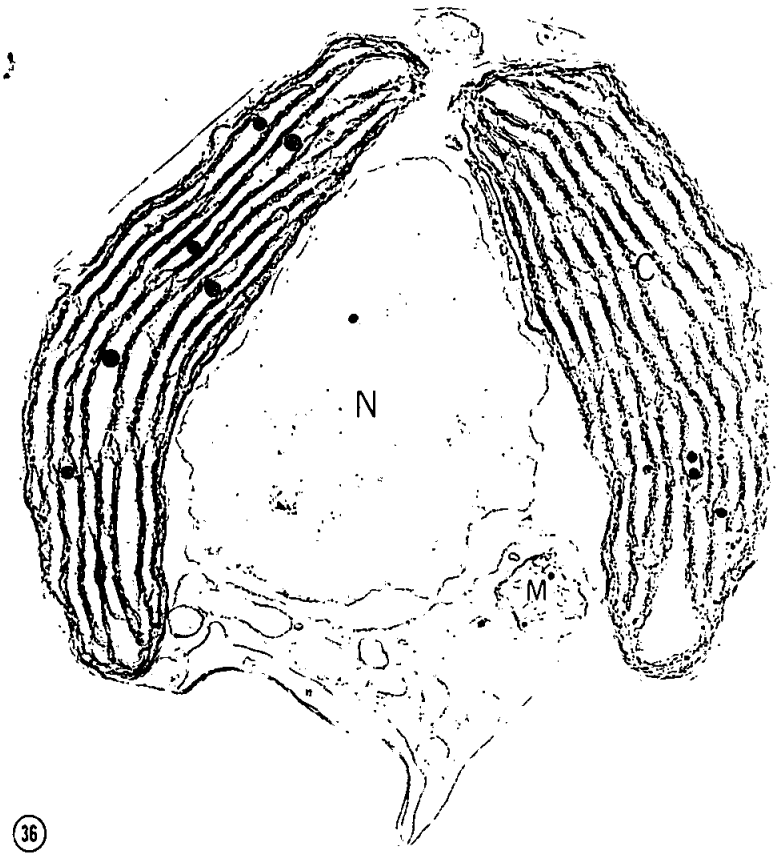
37. Oblique section showing a muciferous body; continuous outer nuclear membrane around the plastid is evident. X 10,500.

38-39. Two different sections showing the presence of two Golgi bodies. X 13,500; X 20,000.

40. Section to show the position of the chrysolaminarin mass at the posterior end of the cell. X 8,500.

41. Two large oil droplets with mitochondria on their surface. X 7,400.

42. Part of a section to show a food vacuole; nuclear pores are evident as are the three limiting membranes (arrows) of the chromatophore. X 10,500.



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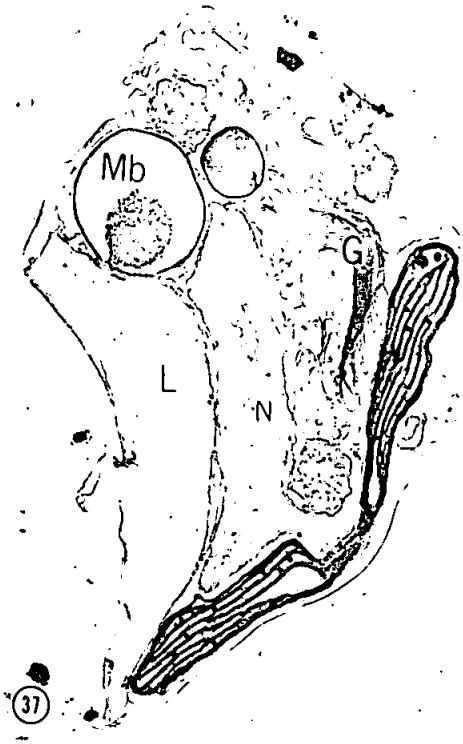

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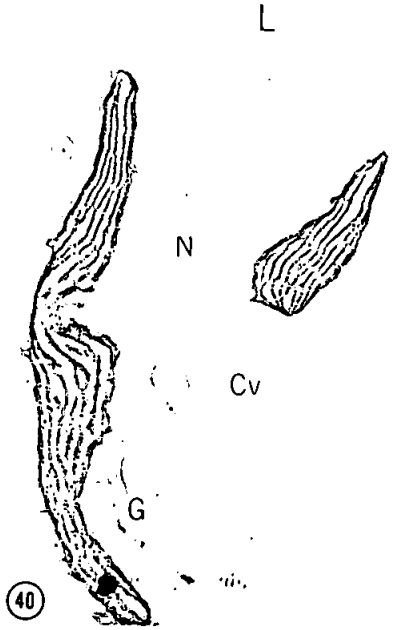

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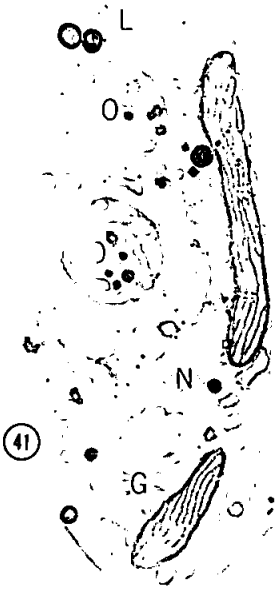

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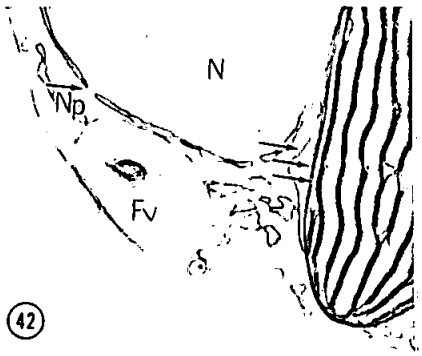

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PLATE VIII.

Figures 43-49, Dinobryon sertularia Ehrenb.

43. Part of a section to show a food vacuole. X 19,500.

44-45. Two sections showing endoplasmic reticulum and possible origin of small vesicles. X 19,000; X 32,000.

46. Light micrograph showing statospores within a colony. X 1,500.

47-48. Two different sections through statospores; a large presence of oil droplets is observed as well as a reduction in the usual number of mitochondria. X 10,300.

49. Part of a section showing vesicles from the Golgi filled with particles that are thought to fuse to form the lorica. X 20,000.

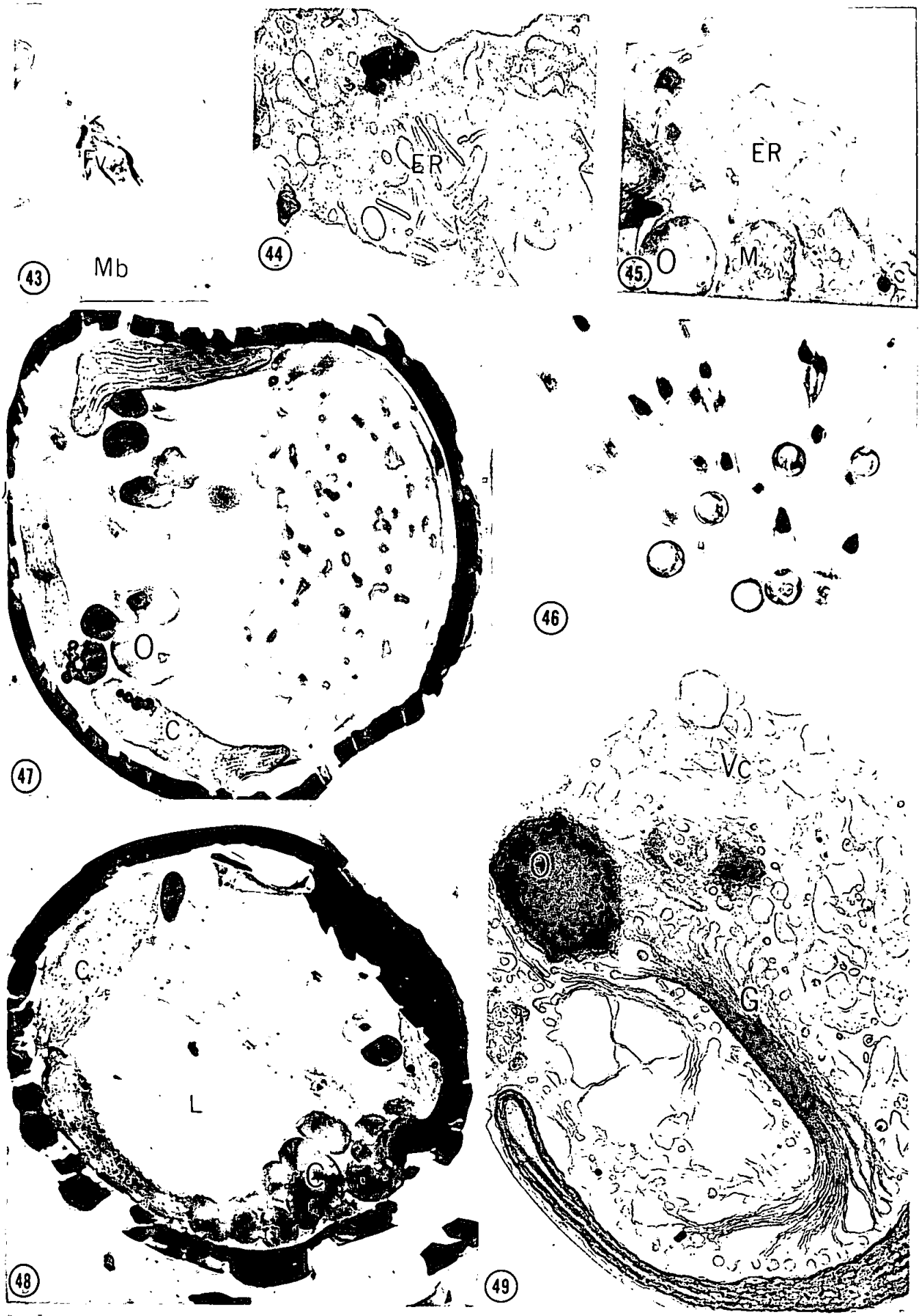

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PLATE IX.

Figures 50-51, Dinobryon sertularia Ehrenb.

50. Part of a section showing vesicles derived from the Golgi filled with particles that fuse to form the lorica. X 16,800.

51. Fibrils (arrow) that make up the lorica. X 46,000.

Figures 52-55, Epipyxis andrewsii sp. nov.

52. Light micrograph. X 1,800.

53. Line drawing of a cyst and loricas.

54. Section of an empty lorica showing scale overlap. X 13,200.

55. Cross section showing the plastid, mitochondria, Golgi and vesicular complex. X 34,000.

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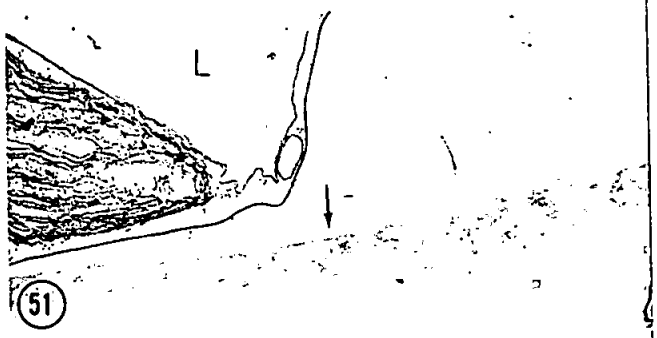

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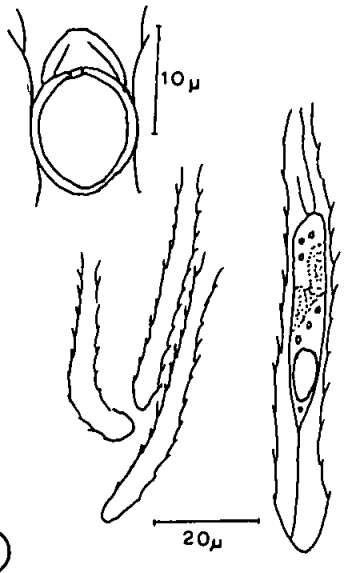

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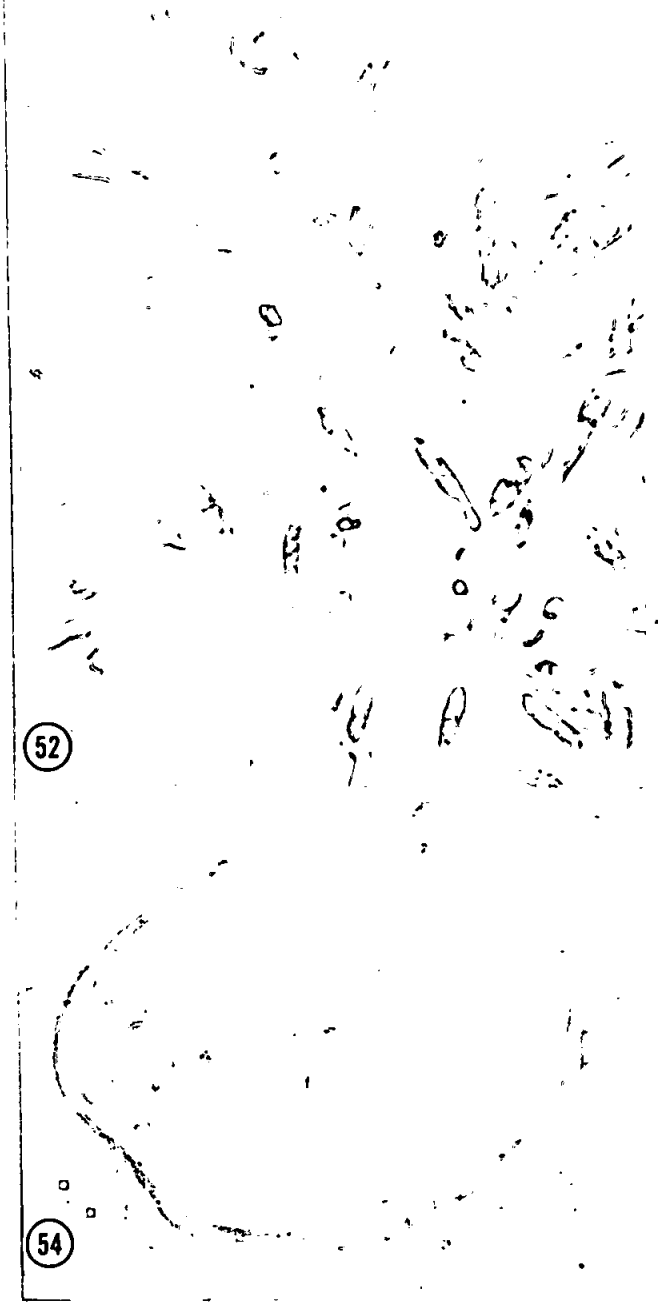

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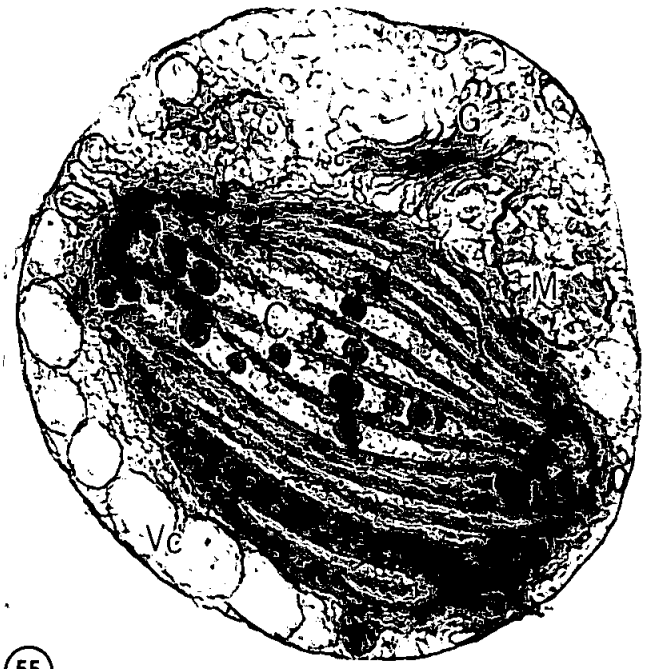

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PLATE X.

Figures 56-59, Epipyxis andrewsii sp. nov.

56. Plastid adjacent to the nucleus. X 17,800.

57. Part of a section showing a Golgi body. X 27,000.

58-58. Large and small oil droplets. X 17,800.

Figure 60, Epipyxis sp.

60. Part of a cell with a food vacuole. X 18,000.

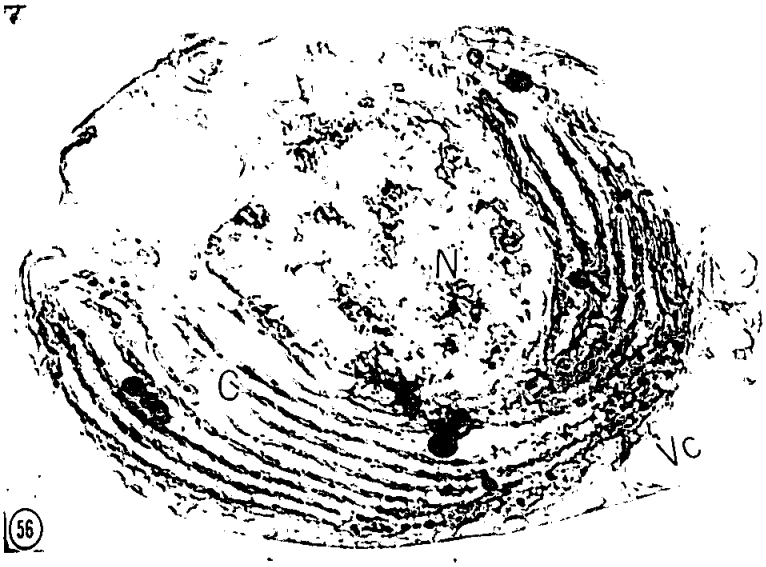
Figures 61-64, Ochromonas thompsonii sp. nov.

61. Diagram of a cyst.

62. Light micrograph of a cyst. X 1,200.

63. Section through a developing plastid. X 14,500.

64. Part of a section through the nucleus and chromatophore; large oil droplets are present. X 20,100.



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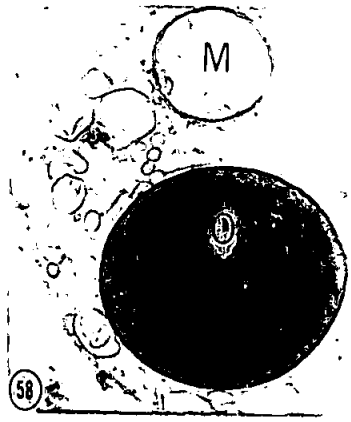

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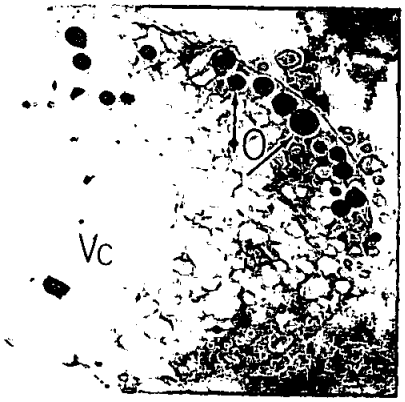

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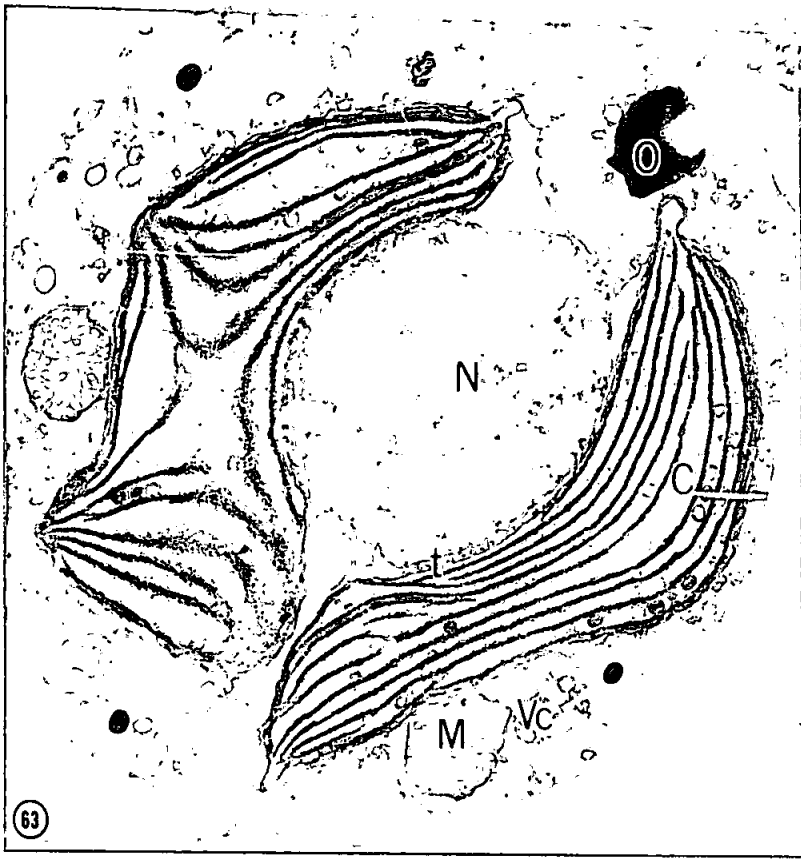

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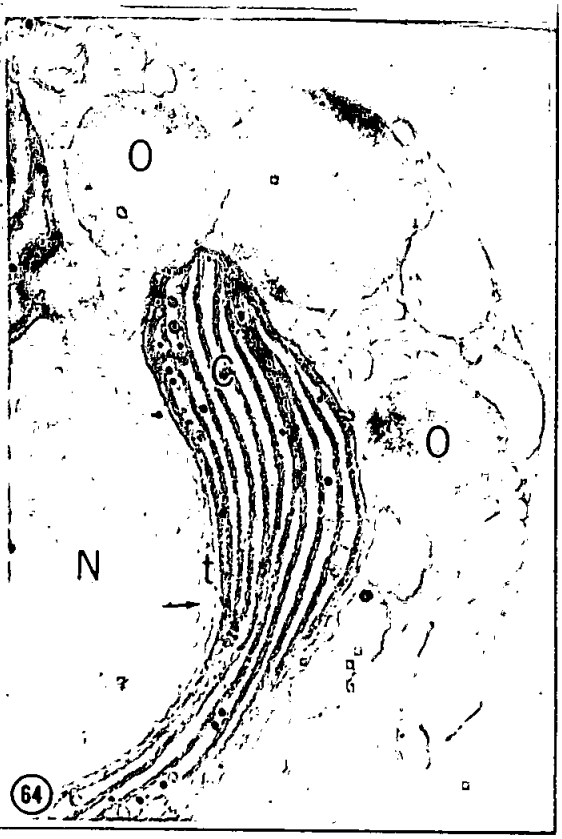

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PLATE XI.

Figures 65-71, Ochromonas thompsonii sp. nov.

64-66. Section through the anterior end of a cell showing a closed and open contractile vacuole. X 21,000.

67. Large oil droplets located about the periphery of the cell. X 18,700.

68. Longitudinal section through a cell showing the chrysolaminarin mass in the posterior end; the outer nuclear membrane is continuous around the plastid which is also limited by two plastid membranes proper; muciferous body is present. X 14,600.

69. Section showing the nucleolus in the posterior end of the nucleus. X 22,100.

70. Oblique section through a cell showing the Golgi body at the anterior end and the vesicles derived from it. X 12,300.

71. Oblique section through the anterior end of a cell showing the association of the flagellar bases and a mitochondrion and Golgi body. X 12,600.


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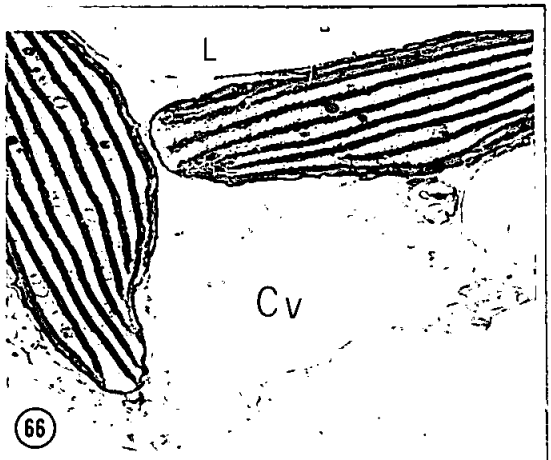
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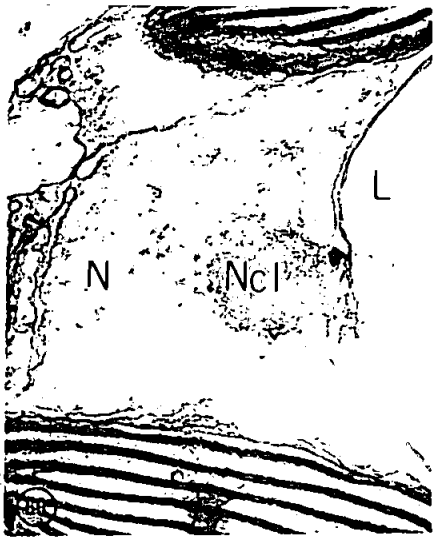
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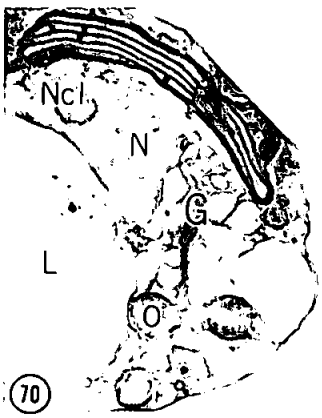
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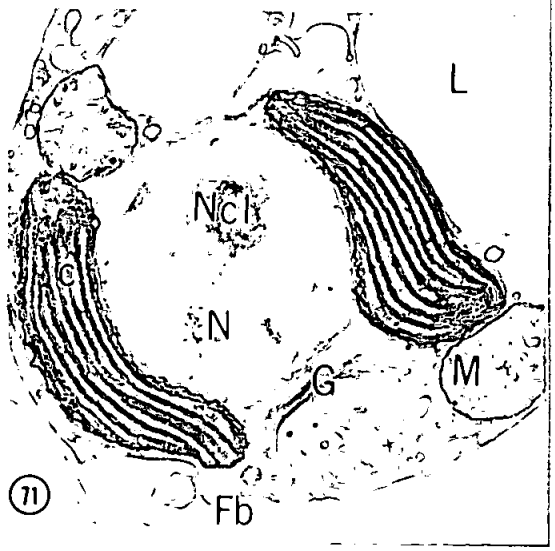
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PLATE XII

Figures 72-76, Ochromonas thompsonii sp. nov.

72. Section showing muciferous body. X 20,000.

73. Section through the chrysolaminarin mass of a cell from a stale culture. X 23,800.

74-76. Three sections through food vacuoles. X 20,000.

Figure 77, Uroglena sp.

77. Light micrograph of a colony. X 2,100.


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PLATE XIII.

Figure 78, Uroglena collaris Thomp.

78. Thin gelatinous threads (arrow) connecting the cells of a colony. X 11,700.

Figures 79-80, Uroglenopsis sp.

79. Section of a flimmer flagellum showing the origin of the mastigonemes from bands (arrow). X 24,000.

80. Broad gelatinous threads (arrow) connecting the cells of a colony. X 9,200.

Figure 81, Uroglenopsis notabilis Mack.

81. Cyst. X 1,800.

Figures 82-83, Uroglena collaris Thomp.

82. Cyst. X 2,000.

83. A whole cell dried down showing flagella lengths. X 16,800.

Figure 84, Uroglenopsis sp.

84. A whole cell dried down to show flagella lengths. X 9,500.

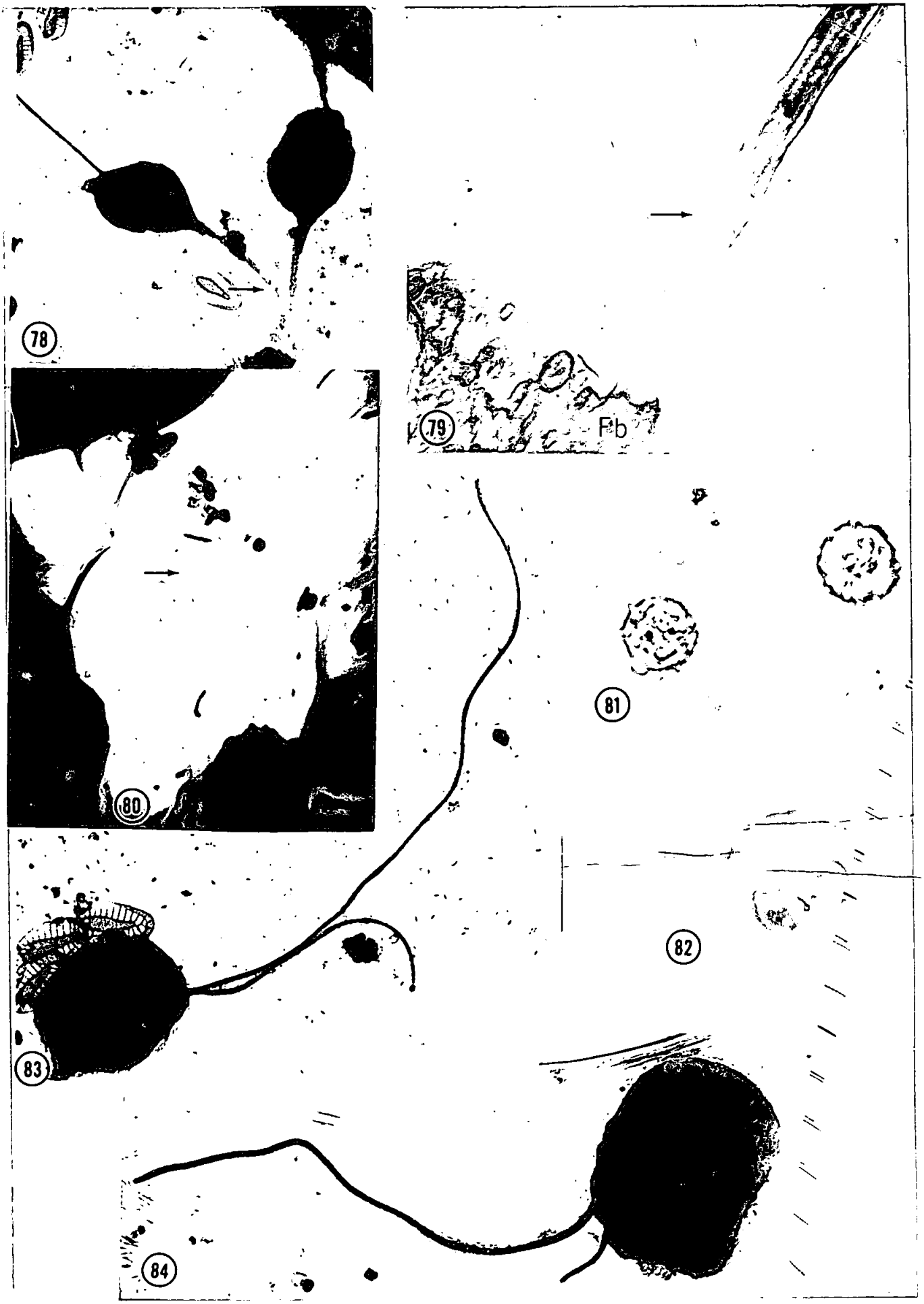
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PLATE XIV.

Figures 85-90, Uroglena and Uroglenopsis spp.

85. Permanganate fixed section showing general cell organelles. X 16,600.

86. Longitudinal section through a cell showing organelles of the cell; pyrenoid is located on the inner surface of the plastid. X 16,300.

87. Diagram of Chrysophyte pyrenoids described to date. a. Hydrurus foetidus. b. Stichochrysis sp. C. Chrysochromulina strobilus. d. C. chiton. e. C. kappa.

88-89. Sections through two mature cells showing the projections of the pyrenoid into the nuclear region. Fig. 88 is permanganate fixed. X 16,000; X 16,400.

90. Cross section through one of the pyrenoid projections found in Fig. 89. X 16,000.

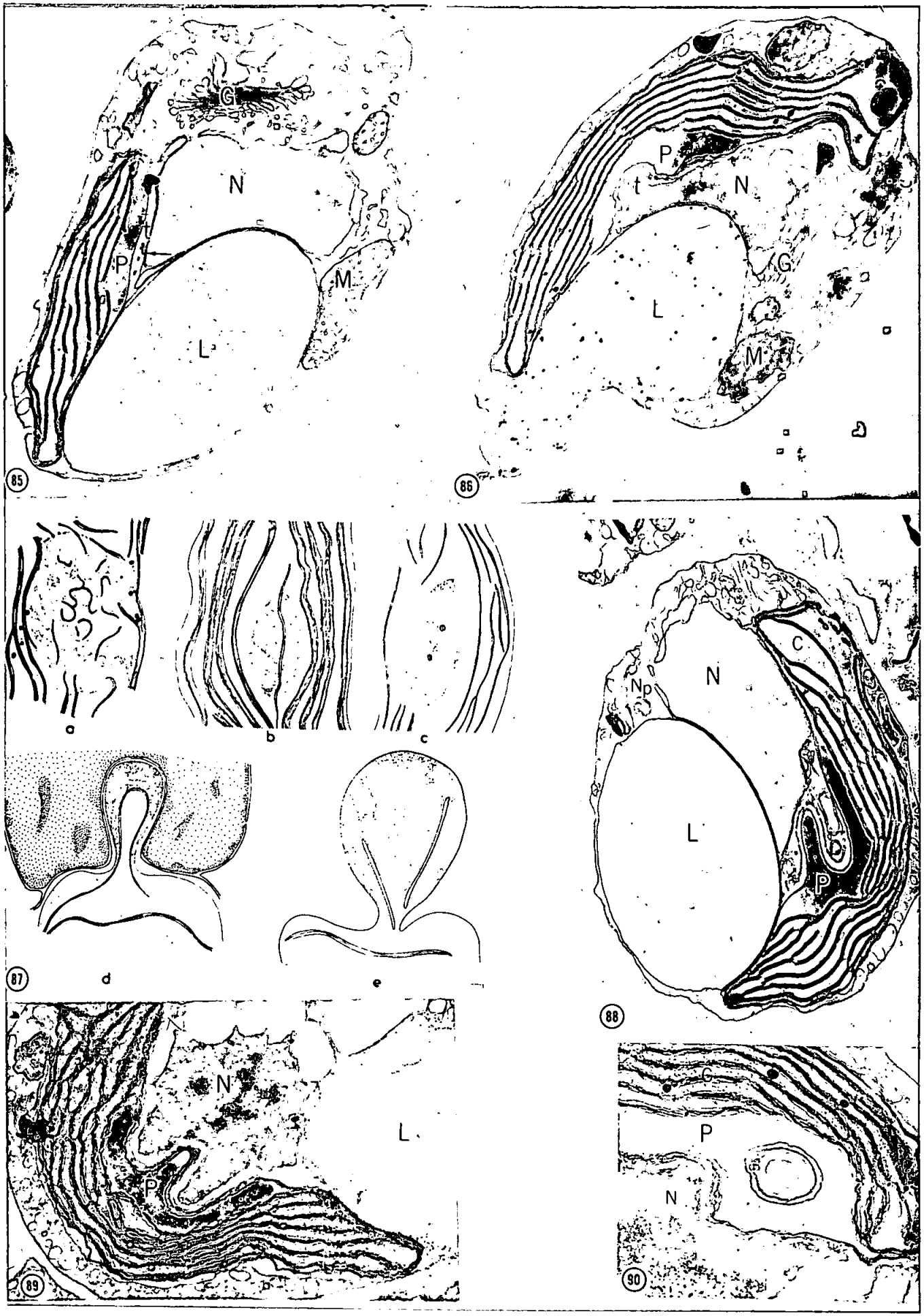
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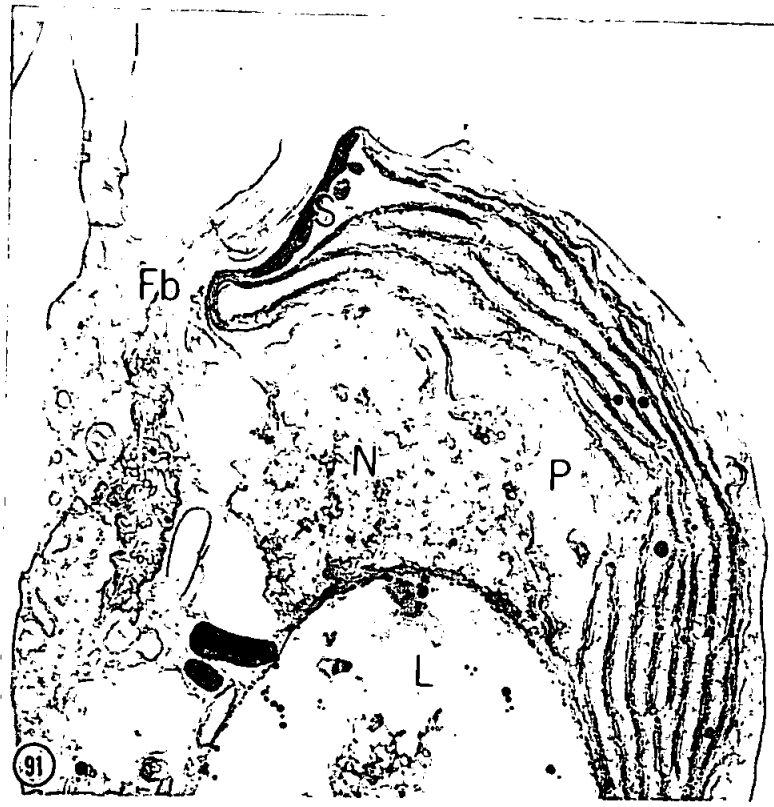
PLATE XV.

Figures 91-94, Uroglena and Uroglenopsis spp.

91. Section showing the whip-lash flagellum adjacent to the eyespot. X 19,000.

92-93. Two sections through two cells showing the double layer of granules and face view of the eyespot. X 17,900; X 21,300.

94. Longitudinal section through a cell showing organelle relationships with the flagellar bases and a root (arrow). X 17,300.



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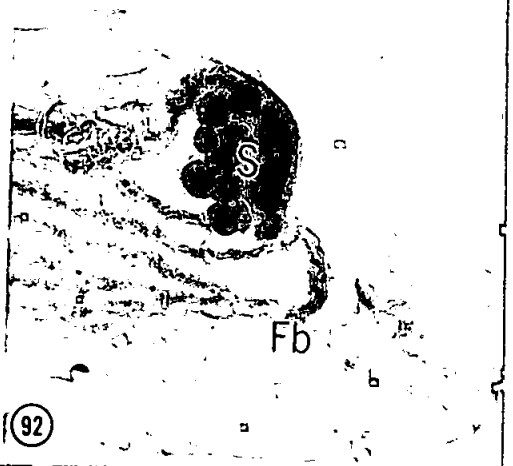

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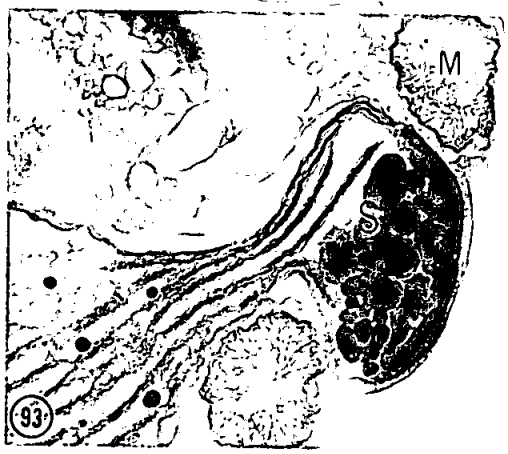

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PLATE XVI.

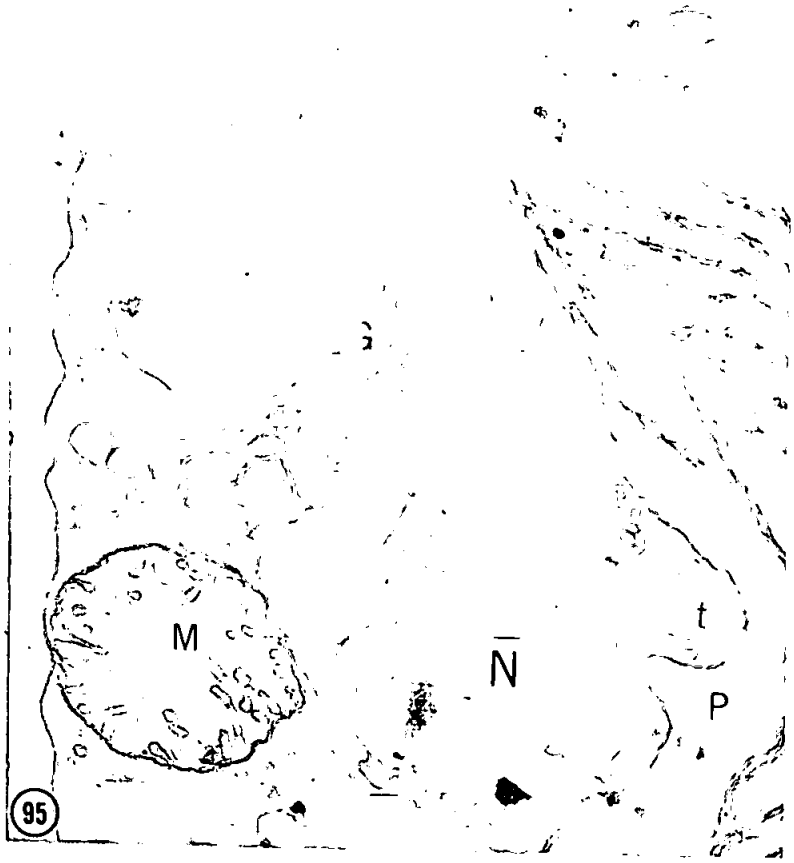
Figures 95-99, Uroglena and Uroglenopsis spp.

95. Part of a section showing a Golgi body in face view with the large vesicles derived from it. X 27,500.

96. Oil droplets at the periphery of the cell. X 21,000.

97. Section of a closed contractile vacuole. X 22,000.

98-99. Sections of Golgi bodies at right angles to that of Fig. 95. X 18,600; X 14,700.



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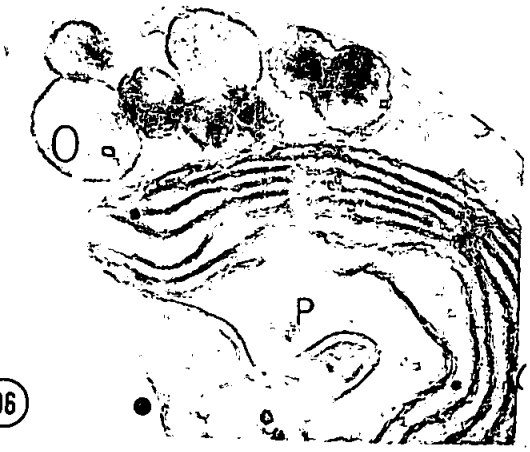

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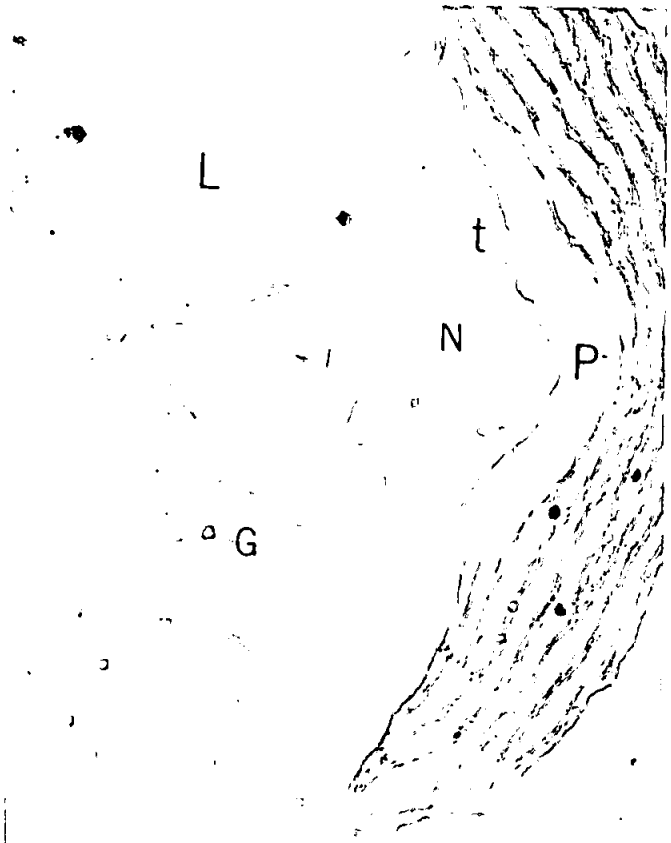

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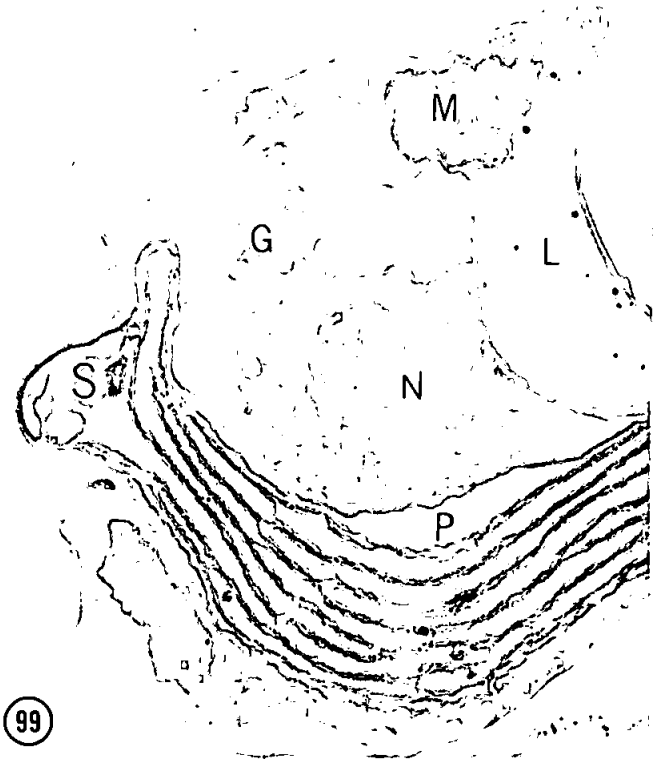

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PLATE XVII.

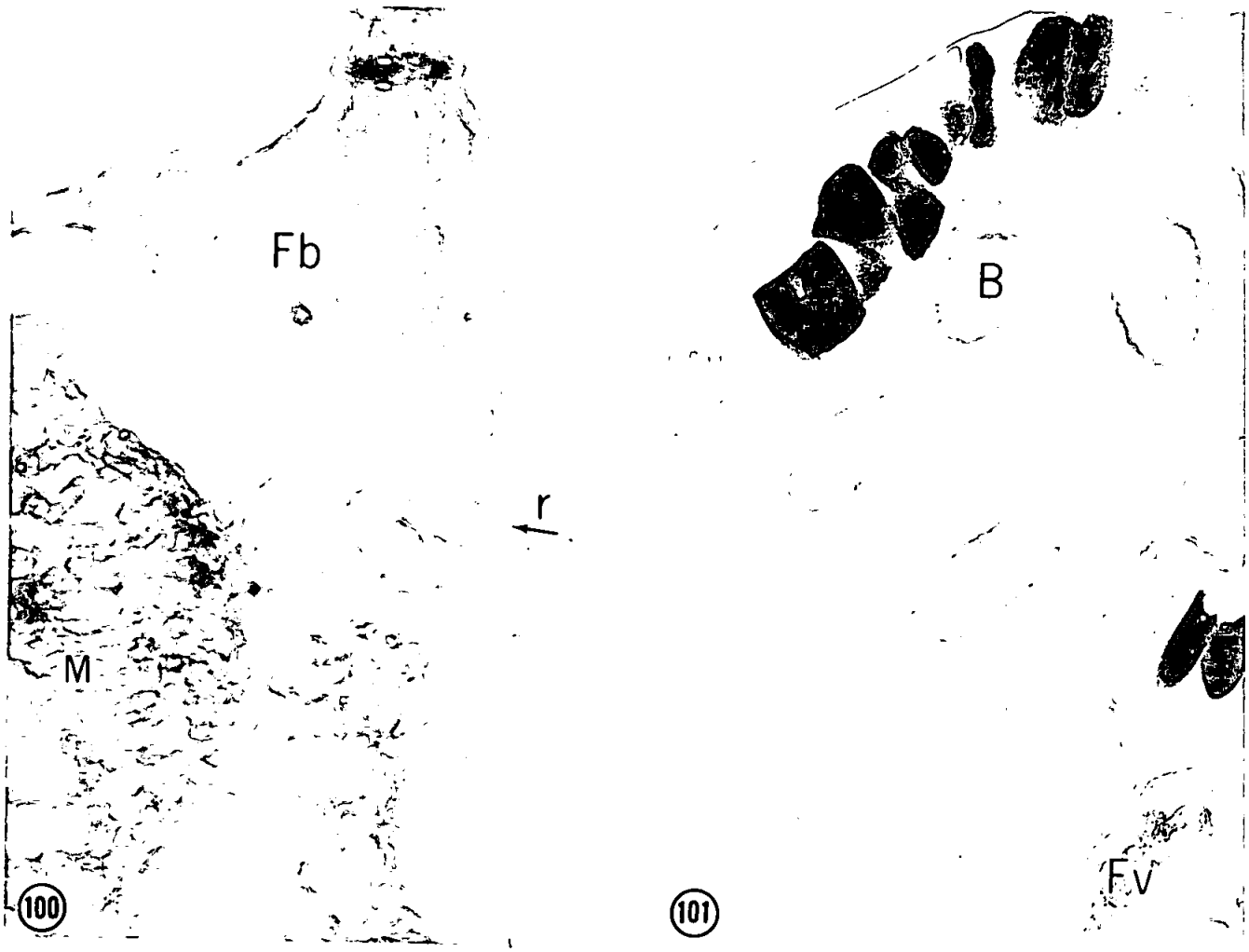
Figures 100-105, Uroglena and Uroglenopsis spp.

100. Section of a flagellum showing the root (arrow). X 18,000.

101. Vesicle containing bacteria. X 24,100.

102-103. Sections of two different food vacuoles. X 24,900; 15,300.

104-105. Sections of two different cells with vesicles (arrows) containing undiscernable bodies. X 10,500.



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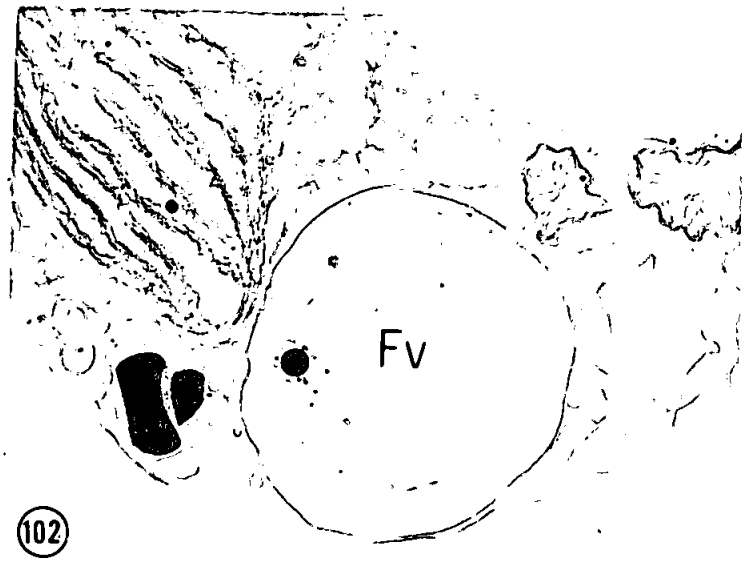

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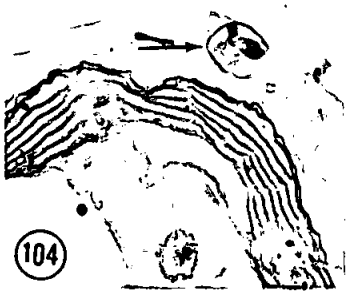

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PLATE XVIII.

Figures 106-112, Uroglena and Uroglenopsis spp.

106. Section of a dividing cell to show the duplicated flagella and their subsequent pulling apart. X 17,500.

107. Section showing the duplicated pyrenoid prior to nuclear division. X 20,000.

108-109. Two different sections of dividing cells showing the duplicated pyrenoids and the beginning of the nuclear events. X 14,700.

110. Part of a section showing the nucleolus reorganizing. X 15,500.

111. Section showing the complete separation of the duplicated plastids and nuclei. X 12,900.


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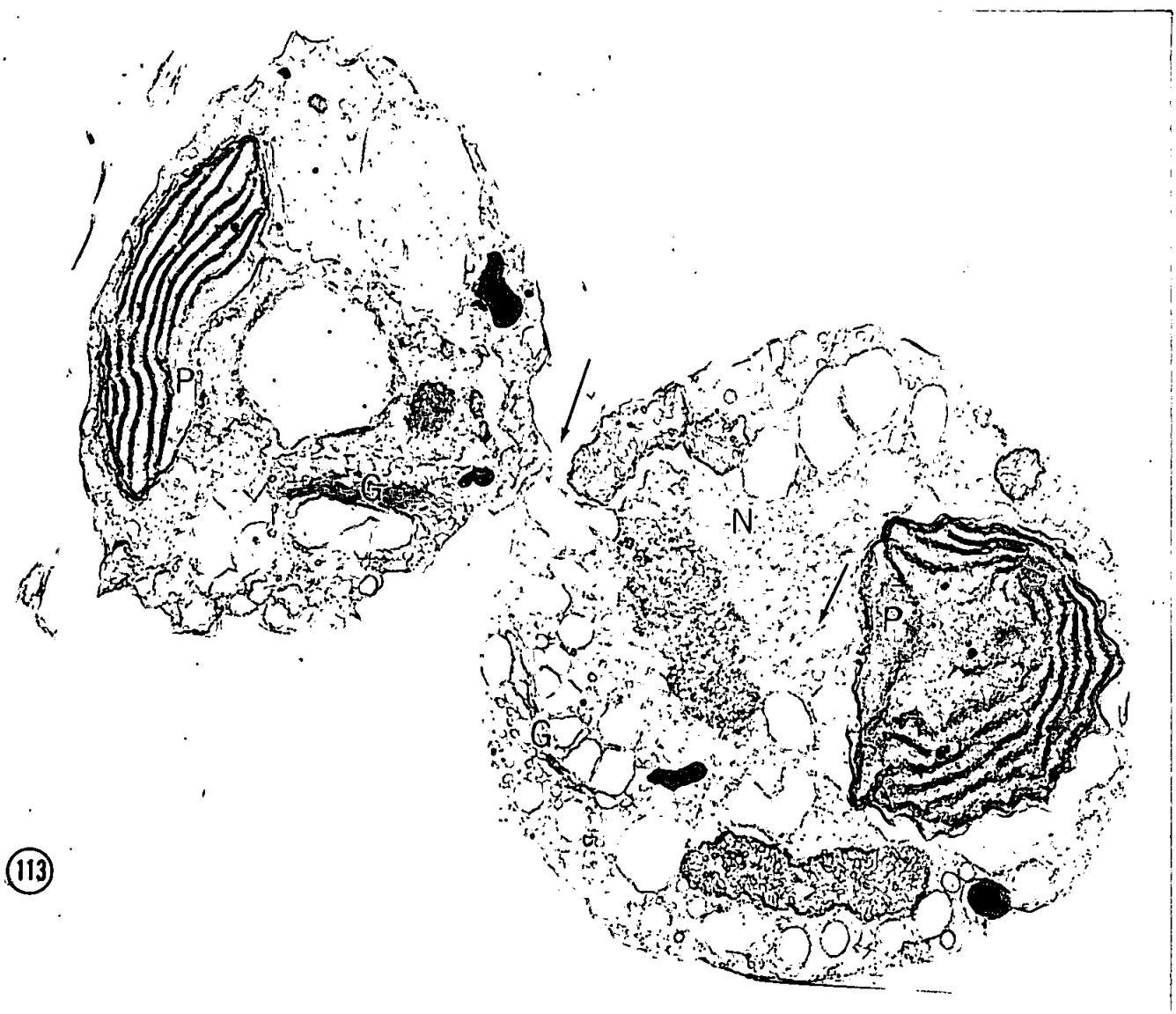
PLATE XIX.

Figures 113-114, Uroglena and Uroglenopsis spp.

113. Section of two cells in the last stage of fission; only a protoplasmic strand (arrow) connects the two daughter cells. X 36,700.

114. Section after the protoplasmic strand has broken to free the two daughter cells. X 21,800.



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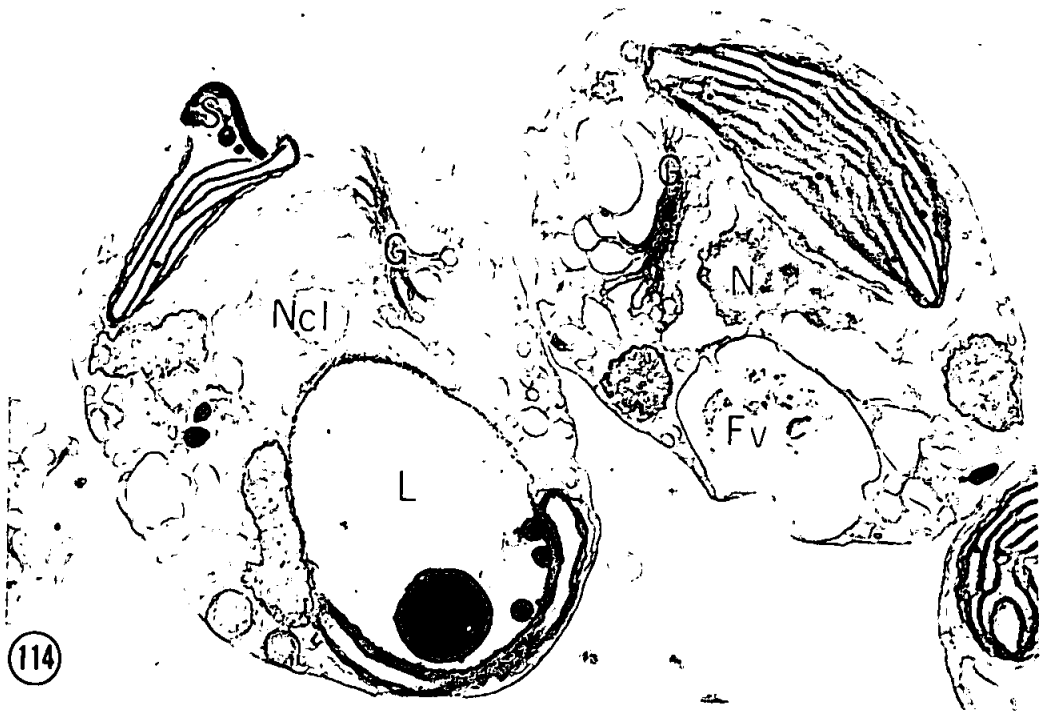
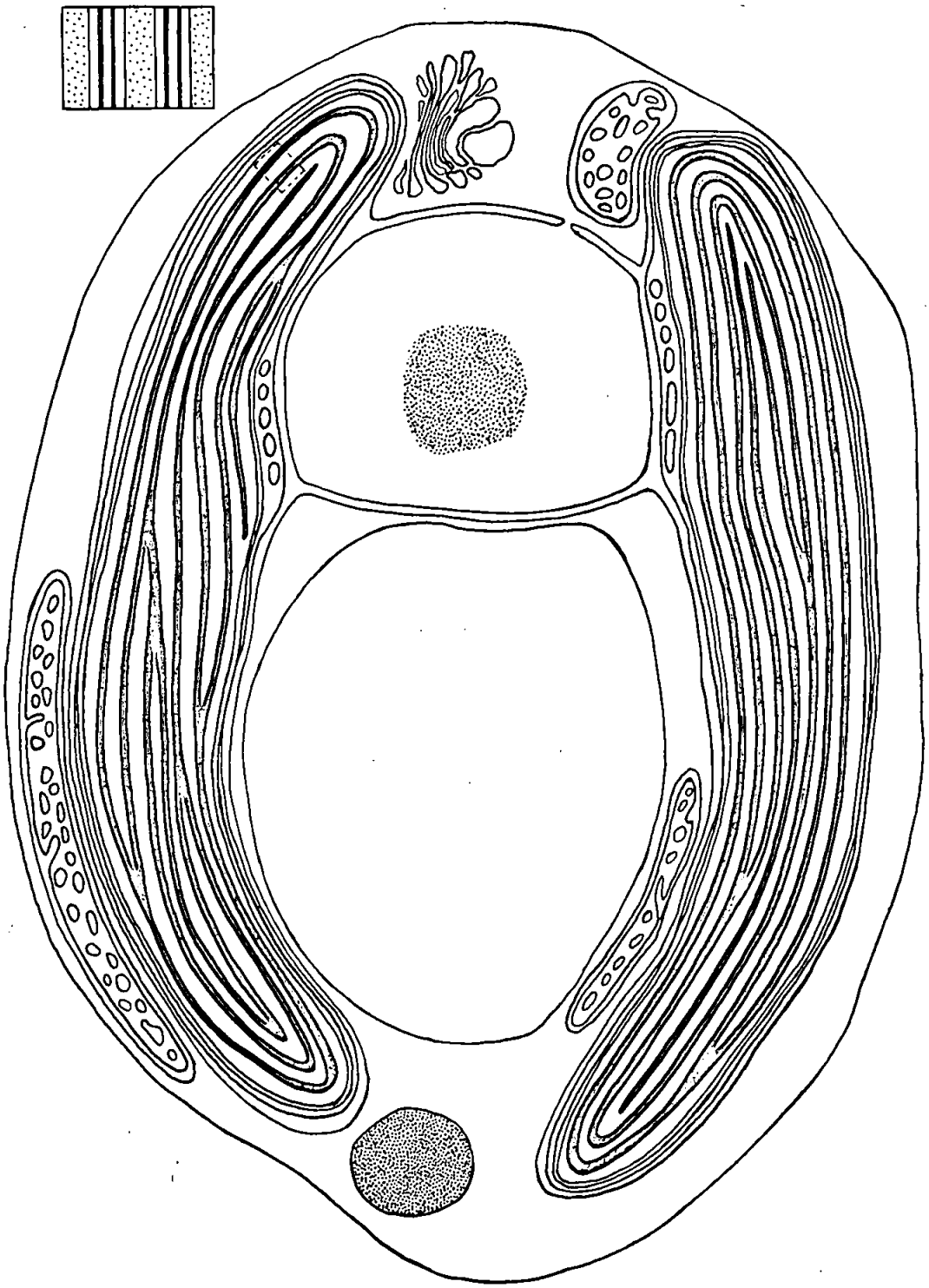


(114)

PLATE XX.

Figure 115. Diagram of a median longitudinal section of a Chrysophyte cell as found in this study. The outer envelope of the chromatophore is seen to be continuous with the outer membrane of the nucleus. Other cell structures shown are the nucleus, oil droplet, mitochondria, the large posterior chrysolaminarin mass, and the tubules which lie in the narrow space between the two membranes which delimit the plastid proper. Details of the plastid lamellae are seen in the upper right hand box.



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